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Date: July 2, 1998

Docket No.: 2121-140P

Assistant Commissioner for Patents
Washington, D.C. 20231

Sir:

This is a Request for filing a ____ continuation X divisional application under 37 C.F.R. § 1.53(b) of pending prior Application No. 08/545,196 filed on October 19, 1995, the entire contents of which are hereby incorporated by reference, by

Judith MELKI and Arnold MUNNICH

for

SPINAL MUSCULAR ATROPHY DIAGNOSTIC METHODS (As Amended)

1. X Enclosed is an application consisting of specification, claims, declaration and drawings/photographs (if applicable).

2. X The filing fee has been calculated as follows:

			LARGE ENTITY	SMALL ENTITY
	BASIC FEE		$790.00	$395.00
	NUMBER FILED	NUMBER EXTRA	RATE FEE	RATE FEE
TOTAL CLAIMS	80- 20 =	60	x 22 = $1,320	x 41 = $
INDEPENDENT CLAIMS	22- 3 =	19	x 82 = $1,558	x 41 = $
_5_ MULTIPLE DEPENDENT CLAIMS PRESENTED			+ $270.00	+ $135.00
		TOTAL	$3,938.00	

3. _X_ A check in the amount of $3,938.00 to cover the filing fee and recording fee (if applicable) is enclosed.

4. ___ Please charge Deposit Account No. 02-2448 in the amount of $__________. A triplicate copy of this request is enclosed.

5. _X_ Amend the specification by inserting before the first line thereof the following:

   a. --This application is a _____ continuation _X_ divisional of copending Application No. 08/545,196, filed on October 19, 1995, the entire contents of which are hereby incorporated by reference.--

   b. --This application is a _____ continuation _____ divisional of copending Application No. __________, filed on ______________________________. Application No. ___________ is the national phase of PCT International Application No. PCT/___/____ filed on __________________________ under 35 U.S.C. § 371. The entire contents of each of the above identified applications are hereby incorporated by reference.--

6. ___ Transfer the drawings/photographs from the prior

application to this application and abandon said prior application as of the filing date accorded this application. A duplicate copy of this request is enclosed for filing in the prior application file.

7. X Enclosed is/are eighteen (18) sheet(s) of formal drawings.

8. ___ A verified statement claiming small entity status was filed in prior Application No. ________ on ________. See attached copy of verified statement claiming small entity.

9. X The prior application is assigned to INSTITUT NATIONAL DE LA SANTE ET DE LA RECHERCHE MEDICALE

10. X A Preliminary Amendment is enclosed.

11a. X Priority of Application No(s). 94402353.0 filed in Europe on October 19, 1994 is/are claimed under 35 U.S.C. § 119. See attached copy of the Letter claiming priority filed in the prior application on February 13, 1996.

11b. ___ Priority of International Appln. ________ filed on ________ under the Patent Cooperation Treaty and ________ Application No. ________ filed in ________ on ________ under 35 U.S.C. § 119 are hereby reclaimed.

12. X An Information Disclosure Statement and PTO-1449 form(s) are attached hereto for the Examiner's consideration.

13. X Address all future communications to:

BIRCH, STEWART, KOLASCH & BIRCH, LLP
P.O. Box 747
Falls Church, Virginia 22040-0747

Telephone: (703) 205-8000

14. ___ An extension of time for ______________ month(s) until ______________ has been submitted in parent Application No. ______________ in order to establish copendency with the present application.

15. X Also enclosed herewith is the following:

Sequence Listing

If necessary, the Commissioner is hereby authorized in this, concurrent, and future replies, to charge payment or credit any overpayment to Deposit Account No. 02-2448 for any additional fees required under 37 C.F.R. § 1.16 or under 37 C.F.R. § 1.17; particularly, extension of time fees.

Respectfully submitted,

BIRCH, STEWART, KOLASCH & BIRCH, LLP

By #32,868

for C. Joseph Faraci
Reg. No. 32,350
P.O. Box 747
Falls Church, VA 22040-0747
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CJF:MAL:jul

Rev. 2/18/98
J:\DIVCONT.7

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT: MELKI, JUDITH
MUNNICH, ARNOLD

(ii) TITLE OF INVENTION: SURVIVAL MOTOR NEURON (SMN) GENE: A GENE FOR SPINAL MUSCULAR ATROPHY

(iii) NUMBER OF SEQUENCES: 57

(iv) CORRESPONDENCE ADDRESS:
(A) ADDRESSEE: BIRCH, STEWART, KOLASCH AND BIRCH, LLP
(B) STREET: PO BOX 747
(C) CITY: FALLS CHURCH
(D) STATE: VA
(E) COUNTRY: USA
(F) ZIP: 22040-0747

(v) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version #1.30

(vi) CURRENT APPLICATION DATA:
(A) APPLICATION NUMBER:
(B) FILING DATE:
(C) CLASSIFICATION:

(viii) ATTORNEY/AGENT INFORMATION:
(A) NAME: FARACI, C. J.
(B) REGISTRATION NUMBER: 32,350
(C) REFERENCE/DOCKET NUMBER: 2121-110P

(ix) TELECOMMUNICATION INFORMATION:
(A) TELEPHONE: (703) 205-8000
(B) TELEFAX: (703) 205-8050

(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 347 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

AATTTTTAAA TTTTTTGTAG AGACAGGGTC TCATTATGTT GCCCAGGGTG GTGTCAAGCT 60

CCAGGTCTCA AGTGATCCCC CTACCTCCGC CTCCCAAAGT TGTGGGATTG TAGGCATGAG 120

CCACTGCAAG AAAACCTTAA CTGCAGCCTA ATAATTGTTT TCTTTGGGAT AACTTTTAAA 180

GTACATTAAA AGACTATCAA CTTAATTTCT GATCATATTT TGTTGAATAA AATAAGTAAA 240

ATGTCTTGTG AACAAAATGC TTTTTAACAT CCATATAAAG CTATCTATAT ATAGCTATCT 300

ATGTCTATAT AGCTATTTTT TTTAACTTCC TTTTATTTTC CTTACAG 347

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 444 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

GTAAGTCTGC CAGCATTATG AAAGTGAATC TTACTTTTGT AAAACTTTAT GGTTTGTGGA 60

AAACAAATGT TTTTGAACAG TTAAAAAGTT CAGATGTTAA AAAGTTGAAA GGTTAATGTA 120

AAACAATCAA TATTAAAGAA TTTTGATGCC AAAACTATTA GATAAAAGGT TAATCTACAT 180

CCCTACTAGA ATTCTCATAC TTAACTGGTT GGTTATGTGG AAGAAACATA CTTTCACAAT 240

AAAGAGCTTT AGGATATGAT GCCATTTTAT ATCACTAGTA GGCAGACCAG CAGACTTTTT 300

TTTATTGTGA TATGGGATAA CCTAGGCATA CTGCACTGTA CACTCTGACA TATGAAGTGC 360

TCTAGTCAAG TTTAACTGGT GTCCACAGAG GACATGGTTT AACTGGAATT CGTCAAGCCT 420

CTGGTTCTAA TTTCTCATTT GCAG 444

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 347 base pairs
(B) TYPE: nucleic acid

(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

```
AATTTTTAAA TTTTTTGTAG AGACAGGGTC TCATTATGTT GCCCAGGGTG GTGTCAAGCT    60
CCAGGTCTCA AGTGATCCCC CTACCTCCGC CTCCCAAAGT TGTGGGATTG TAGGCATGAG   120
CCACTGCAAG AAAACCTTAA CTGCAGCCTA ATAATTGTTT TCTTTGGGAT AACTTTTAAA   180
GTACATTAAA AGACTATCAA CTTAATTTCT GATCATATTT TGTTGAATAA AATAAGTAAA   240
ATGTCTTGTG AACAAAATGC TTTTTAACAT CCATATAAAG CTATCTATAT ATAGCTATCT   300
ATATCTATAT AGCTATTTTT TTTAACTTCC TTTTATTTTC CTTACAG                347
```

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 444 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

```
GTAAGTCTGC CAGCATTATG AAAGTGAATC TTACTTTTGT AAAACTTTAT GGTTTGTGGA    60
AAACAAATGT TTTTGAACAG TTAAAAAGTT CAGATGTTAG AAAGTTGAAA GGTTAATGTA   120
AAACAATCAA TATTAAAGAA TTTTGATGCC AAAACTATTA GATAAAAGGT TAATCTACAT   180
CCCTACTAGA ATTCTCATAC TTAACTGGTT GGTTGTGTGG AAGAAACATA CTTTCACAAT   240
AAAGAGCTTT AGGATATGAT GCCATTTTAT ATCACTAGTA GGCAGACCAG CAGACTTTTT   300
TTTATTGTGA TATGGGATAA CCTAGGCATA CTGCACTGTA CACTCTGACA TATGAAGTGC   360
TCTAGTCAAG TTTAACTGGT GTCCACAGAG GACATGGTTT AACTGGAATT CGTCAAGCCT   420
CTGGTTCTAA TTTCTCATTT GCAG                                         444
```

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 25 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc = "SYNTHETIC DNA"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

AGACTATCAA CTTAATTTCT GATCA 25

(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 24 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc = "SYNTHETIC DNA"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

TAAGGAATGT GAGCACCTTC CTTC 24

(2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 23 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc = "SYNTHETIC DNA"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

GTAATAACCA AATGCAATGT GAA 23

(2) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc = "SYNTHETIC DNA"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

CTACAACACC CTTCTCACAG 20

(2) INFORMATION FOR SEQ ID NO:9:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 294 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

```
Met Ala Met Ser Ser Gly Gly Ser Gly Gly Gly Val Pro Glu Gln Glu
1               5                   10                  15

Asp Ser Val Leu Phe Arg Arg Gly Thr Gly Gln Ser Asp Asp Ser Asp
            20                  25                  30

Ile Trp Asp Asp Thr Ala Leu Ile Lys Ala Tyr Asp Lys Ala Val Ala
        35                  40                  45

Ser Phe Lys His Ala Leu Lys Asn Gly Asp Ile Cys Glu Thr Ser Gly
    50                  55                  60

Lys Pro Lys Thr Thr Pro Lys Arg Lys Pro Ala Lys Lys Asn Lys Ser
65                  70                  75                  80

Gln Lys Lys Asn Thr Ala Ala Ser Leu Gln Gln Trp Lys Val Gly Asp
                85                  90                  95
```

Lys Cys Ser Ala Ile Trp Ser Glu Asp Gly Cys Ile Tyr Pro Ala Thr
100 105 110

Ile Ala Ser Ile Asp Phe Lys Arg Glu Thr Cys Val Val Val Tyr Thr
115 120 125

Gly Tyr Gly Asn Arg Glu Glu Gln Asn Leu Ser Asp Leu Leu Ser Pro
130 135 140

Ile Cys Glu Val Ala Asn Asn Ile Glu Gln Asn Ala Gln Glu Asn Glu
145 150 155 160

Asn Glu Ser Gln Val Ser Thr Asp Glu Ser Glu Asn Ser Arg Ser Pro
165 170 175

Gly Asn Lys Ser Asp Asn Ile Lys Pro Lys Ser Ala Pro Trp Asn Ser
180 185 190

Phe Leu Pro Pro Pro Pro Pro Met Pro Gly Pro Arg Leu Gly Pro Gly
195 200 205

Lys Pro Gly Leu Lys Phe Asn Gly Pro Pro Pro Pro Pro Pro Pro Pro
210 215 220

Pro Pro His Leu Leu Ser Cys Trp Leu Pro Pro Phe Pro Ser Gly Pro
225 230 235 240

Pro Ile Ile Pro Pro Pro Pro Pro Ile Cys Pro Asp Ser Leu Asp Asp
245 250 255

Ala Asp Ala Leu Gly Ser Met Leu Ile Ser Trp Tyr Met Ser Gly Tyr
260 265 270

His Thr Gly Tyr Tyr Met Gly Phe Arg Gln Asn Gln Lys Glu Gly Arg
275 280 285

Cys Ser His Ser Leu Asn
290

(2) INFORMATION FOR SEQ ID NO:10:

(i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 1582 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

```
CGGGGCCCCA CGCTGCGCAC CCGCGGGTTT GCTATGGCGA TGAGCAGCGG CGGCAGTGGT      60
GGCGGCGTCC CGGAGCAGGA GGATTCCGTG CTGTTCCGGC GCGGCACAGG CCAGAGCGAT     120
GATTCTGACA TTTGGGATGA TACAGCACTG ATAAAAGCAT ATGATAAAGC TGTGGCTTCA     180
TTTAAGCATG CTCTAAAGAA TGGTGACATT TGTGAAACTT CGGGTAAACC AAAAACCACA     240
CCTAAAAGAA AACCTGCTAA GAAGAATAAA AGCCAAAAGA AGAATACTGC AGCTTCCTTA     300
CAACAGTGGA AAGTTGGGGA CAAATGTTCT GCCATTTGGT CAGAAGACGG TTGCATTTAC     360
CCAGCTACCA TTGCTTCAAT TGATTTTAAG AGAGAAACCT GTGTTGTGGT TTACACTGGA     420
TATGGAAATA GAGAGGAGCA AAATCTGTCC GATCTACTTT CCCCAATCTG TGAAGTAGCT     480
AATAATATAG AACAGAATGC TCAAGAGAAT GAAAATGAAA GCCAAGTTTC AACAGATGAA     540
AGTGAGAACT CCAGGTCTCC TGGAAATAAA TCAGATAACA TCAAGCCCAA ATCTGCTCCA     600
TGGAACCCCT TTCTCCCTCC ACCACCCCCC ATGCCAGGGC CAAGACTGGG ACCAGGAAAG     660
CCAGGTCTAA AATTCAATGG CCCACCACCG CCACCGCCAC CACCACCACC CCACTTACTA     720
TCATGCTGGC TGCCTCCATT TCCTTCTGGA CCACCAATAA TTCCCCCACC ACCTCCCATA     780
TGTCCAGATT CTCTTGATGA TGCTGATGCT TTGGGAAGTA TGTTAATTTC ATGGTACATG     840
AGTGGCTATC ATACTGGCTA TTATATGGGT TTTAGACAAA ATCAAAAAGA AGGAAGGTGC     900
TCACATTCCT TAAATTAAGG AGAAATGCTG GCATAGAGCA GCACTAAATG ACACCACTAA     960
AGAAACGATC AGACAGATCT GGAATGTGAA GCGTTATAGA AGATAACTGG CCTCATTTCT    1020
TCAAAATATC AAGTGTTGGG AAAGAAAAAA GGAAGTGGAA TGGGTAACTC TTCTTGATTA    1080
AAAGTTATGT AATAACCAAA TGCAATGTGA AATATTTTAC TGGACTCTTT TGAAAAACCA    1140
TCTGTAAAAG ACTGAGGTGG GGGTGGGAGG CCAGCACGGT GGTGAGGCAG TTGAGAAAAT    1200
TTGAATGTGG ATTAGATTTT GAATGATATT GGATAATTAT TGGTAATTTT ATGGCCTGTG    1260
AGAAGGGTGT TGTAGTTTAT AAAAGACTGT CTTAATTTGC ATACTTAAGC ATTTAGGAAT    1320
GAAGTGTTAG AGTGTCTTAA AATGTTTCAA ATGGTTTAAC AAAATGTATG TGAGGCGTAT    1380
GTGGCAAAAT GTTACAGAAT CTAACTGGTG GACATGGCTG TTCATTGTAC TGTTTTTTTC    1440
TATCTTCTAT ATGTTTAAAA GTATATAATA AAAATATTTA ATTTTTTTTT AAAAAAAAAA    1500
```

AAAAAAAAAA AAAAAAAAAA AAAAAAAAAA AAAAAAAAAA AAAAAAAAAA AAAAAAAAAA 1560

AAAAAAAAAA AAAAAAAAAA AA 1582

(2) INFORMATION FOR SEQ ID NO:11:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1408 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

AATTTTTAAA TTTTTTGTAG AGACAGGGTC TCATTATGTT GCCCAGGGTG GTGTCAAGCT 60

CCAGGTCTCA AGTGATCCCC CTACCTCCGC CTCCCAAAGT TGTGGGATTG TAGGCATGAG 120

CCACTGCAAG AAAACCTTAA CTGCAGCCTA ATAATTGTTT TCTTTGGGAT AACTTTTAAA 180

GTACATTAAA AGACTATCAA CTTAATTTCT GATCATATTT TGTTGAATAA AATAAGTAAA 240

ATGTCTTGTG AACAAAATGC TTTTTAACAT CCATATAAAG CTATCTATAT ATAGCTATCT 300

ATATCTATAT AGCTATTTTT TTTAACTTCC TTTTATTTTC CTTACAGGGT TTTAGACAAA 360

ATCAAAAAGA AGGAAGGTGC TCACATTCCT TAAATTAAGG AGTAAGTCTG CCAGCATTAT 420

GAAAGTGAAT CTTACTTTTG TAAAACTTTA TGGTTTGTGG AAAACAAATG TTTTTGAACA 480

GTTAAAAAGT TCAGATGTTA GAAAGTTGAA AGGTTAATGT AAAACAATCA ATATTAAAGA 540

ATTTTGATGC CAAAACTATT AGATAAAAGG TTAATCTACA TCCCTACTAG AATTCTCATA 600

CTTAACTGGT TGGTTGTGTG GAAGAAACAT ACTTTCACAA TAAAGAGCTT TAGGATATGA 660

TGCCATTTTA TATCACTAGT AGGCAGACCA GCAGACTTTT TTTTATTGTG ATATGGGATA 720

ACCTAGGCAT ACTGCACTGT ACACTCTGAC ATATGAAGTG CTCTAGTCAA GTTTAACTGG 780

TGTCCACAGA GGACATGGTT TAACTGGAAT TCGTCAAGCC TCTGGTTCTA ATTTCTCATT 840

TGCAGGAAAT GCTGGCATAG AGCAGCACTA AATGACACCA CTAAAGAAAC GATCAGACAG 900

ATCTGGAATG TGAAGCGTTA TAGAAGATAA CTGGCCTCAT TTCTTCAAAA TATCAAGTGT 960

TGGGAAAGAA AAAAGGAAGT GGAATGGGTA ACTCTTCTTG ATTAAAAGTT ATGTAATAAC 1020

CAAATGCAAT GTGAAATATT TTACTGGACT CTTTTGAAAA ACCATCTGTA AAAGACTGAG 1080

GTGGGGGTGG GAGGCCAGCA CGGTGGTGAG GCAGTTGAGA AAATTTGAAT GTGGATTAGA 1140

TTTTGAATGA TATTGGATAA TTATTGGTAA TTTTATGGCC TGTGAGAAGG GTGTTGTAGT 1200

TTATAAAAGA CTGTCTTAAT TTGCATACTT AAGCATTTAG GAATGAAGTG TTAGAGTGTC 1260

TTAAAATGTT TCAAATGGTT TAACAAAATG TATGTGAGGC GTATGTGGCA AAATGTTACA 1320

GAATCTAACT GGTGGACATG GCTGTTCATT GTACTGTTTT TTTCTATCTT CTATATGTTT 1380

AAAAGTATAT AATAAAAATA TTTAATTT 1408

(2) INFORMATION FOR SEQ ID NO:12:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1582 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

CGGGGCCCCA CGCTGCGCAT CCGCGGGTTT GCTATGGCGA TGAGCAGCGG CGGCAGTGGT 60

GGCGGCGTCC CGGAGCAGGA GGATTCCGTG CTGTTCCGGC GCGGCACAGG CCAGAGCGAT 120

GATTCTGACA TTTGGGATGA TACAGCACTG ATAAAAGCAT ATGATAAAGC TGTGGCTTCA 180

TTTAAGCATG CTCTAAAGAA TGGTGACATT TGTGAAACTT CGGGTAAACC AAAAACCACA 240

CCTAAAAGAA AACCTGCTAA GAAGAATAAA AGCCAAAAGA AGAATACTGC AGCTTCCTTA 300

CAACAGTGGA AAGTTGGGGA CAAATGTTCT GCCATTTGGT CAGAAGACGG TTGCATTTAC 360

CCAGCTACCA TTGCTTCAAT TGATTTTAAG AGAGAAACCT GTGTTGTGGT TTACACTGGA 420

TATGGAAATA GAGAGGAGCA AAATCTGTCC GATCTACTTT CCCCAATCTG TGAAGTAGCT 480

AATAATATAG AACAGAATGC TCAAGAGAAT GAAAATGAAA GCCAAGTTTC AACAGATGAA 540

AGTGAGAACT CCAGGTCTCC TGGAAATAAA TCAGATAACA TCAAGCCCAA ATCTGCTCCA 600

TGGAACTCTT TTCTCCCTCC ACCACCCCCC ATGCCAGGGC CAAGACTGGG ACCAGGAAAG 660

CCAGGTCTAA AATTCAATGG CCCACCACCG CCACCGCCAC CACCACCACC CCACTTACTA 720

TCATGCTGGC TGCCTCCATT TCCTTCTGGA CCACCAATAA TTCCCCCACC ACCTCCCATA 780

TGTCCAGATT CTCTTGATGA TGCTGATGCT TTGGGAAGTA TGTTAATTTC ATGGTACATG 840

AGTGGCTATC ATACTGGCTA TTATATGGGT TTCAGACAAA ATCAAAAAGA AGGAAGGTGC 900

TCACATTCCT TAAATTAAGG AGAAATGCTG GCATAGAGCA GCACTAAATG ACACCACTAA 960

AGAAACGATC AGACAGATCT GGAATGTGAA GCGTTATAGA AGATAACTGG CCTCATTTCT 1020

TCAAAATATC AAGTGTTGGG AAAGAAAAAA GGAAGTGGAA TGGGTAACTC TTCTTGATTA 1080

AAAGTTATGT AATAACCAAA TGCAATGTGA AATATTTTAC TGGACTCTTT TGAAAAACCA 1140

TCTGTAAAAG ACTGGGGTGG GGGTGGGAGG CCAGCACGGT GGTGAGGCAG TTGAGAAAAT 1200

TTGAATGTGG ATTAGATTTT GAATGATATT GGATAATTAT TGGTAATTTT ATGGCCTGTG 1260

AGAAGGGTGT TGTAGTTTAT AAAAGACTGT CTTAATTTGC ATACTTAAGC ATTTAGGAAT 1320

GAAGTGTTAG AGTGTCTTAA AATGTTTCAA ATGGTTTAAC AAAATGTATG TGAGGCGTAT 1380

GTGGCAAAAT GTTACAGAAT CTAACTGGTG GACATGGCTG TTCATTGTAC TGTTTTTTTC 1440

TATCTTCTAT ATGTTTAAAA GTATATAATA AAAATATTTA ATTTTTTTTT AAAAAAAAAA 1500

AAAAAAAAAA AAAAAAAAAA AAAAAAAAAA AAAAAAAAAA AAAAAAAAAA AAAAAAAAAA 1560

AAAAAAAAAA AAAAAAAAAA AA 1582

(2) INFORMATION FOR SEQ ID NO:13:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1408 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

AATTTTTAAA TTTTTTGTAG AGACAGGGTC TCATTATGTT GCCCAGGGTG GTGTCAAGCT 60

CCAGGTCTCA AGTGATCCCC CTACCTCCGC CTCCCAAAGT TGTGGGATTG TAGGCATGAG 120

CCACTGCAAG AAAACCTTAA CTGCAGCCTA ATAATTGTTT TCTTTGGGAT AACTTTTAAA 180

GTACATTAAA AGACTATCAA CTTAATTTCT GATCATATTT TGTTGAATAA AATAAGTAAA 240

ATGTCTTGTG AACAAAATGC TTTTTAACAT CCATATAAAG CTATCTATAT ATAGCTATCT 300

ATGTCTATAT AGCTATTTTT TTTAACTTCC TTTTATTTTC CTTACAGGGT TTCAGACAAA 360

ATCAAAAAGA AGGAAGGTGC TCACATTCCT TAAATTAAGG AGTAAGTCTG CCAGCATTAT 420

GAAAGTGAAT CTTACTTTTG TAAAACTTTA TGGTTTGTGG AAAACAAATG TTTTTGAACA 480

GTTAAAAAGT TCAGATGTTA AAAAGTTGAA AGGTTAATGT AAAACAATCA ATATTAAAGA 540

ATTTTGATGC CAAAACTATT AGATAAAAGG TTAATCTACA TCCCTACTAG AATTCTCATA 600

CTTAACTGGT TGGTTATGTG GAAGAAACAT ACTTTCACAA TAAAGAGCTT TAGGATATGA 660

TGCCATTTTA TATCACTAGT AGGCAGACCA GCAGACTTTT TTTTATTGTG ATATGGGATA 720

ACCTAGGCAT ACTGCACTGT ACACTCTGAC ATATGAAGTG CTCTAGTCAA GTTTAACTGG 780

TGTCCACAGA GGACATGGTT TAACTGGAAT TCGTCAAGCC TCTGGTTCTA ATTTCTCATT 840

TGCAGGAAAT GCTGGCATAG AGCAGCACTA AATGACACCA CTAAAGAAAC GATCAGACAG 900

ATCTGGAATG TGAAGCGTTA TAGAAGATAA CTGGCCTCAT TTCTTCAAAA TATCAAGTGT 960

TGGGAAAGAA AAAAGGAAGT GGAATGGGTA ACTCTTCTTG ATTAAAAGTT ATGTAATAAC 1020

CAAATGCAAT GTGAAATATT TTACTGGACT CTTTTGAAAA ACCATCTGTA AAAGACTGGG 1080

GTGGGGGTGG GAGGCCAGCA CGGTGGTGAG GCAGTTGAGA AAATTTGAAT GTGGATTAGA 1140

TTTTGAATGA TATTGGATAA TTATTGGTAA TTTTATGGCC TGTGAGAAGG GTGTTGTAGT 1200

TTATAAAAGA CTGTCTTAAT TTGCATACTT AAGCATTTAG GAATGAAGTG TTAGAGTGTC 1260

TTAAAATGTT TCAAATGGTT TAACAAAATG TATGTGAGGC GTATGTGGCA AAATGTTACA 1320

GAATCTAACT GGTGGACATG GCTGTTCATT GTACTGTTTT TTTCTATCTT CTATATGTTT 1380

AAAAGTATAT AATAAAAATA TTTAATTT 1408

(2) INFORMATION FOR SEQ ID NO:14:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 360 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc = "SYNTHETIC DNA"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:

ACCTGACCCA GAGGTCAAGG CTGCAGTGAG ACGAGATTGC CCACTGCCCT CCACCCTGGG 60

TGATAAGAGT GGGACCCTGT TCAAAACATA CACACACACA CACACACACA CACACACACA 120

CACACACACA CTCTCTCTCT CTCTCTCTCT CTCTCTCTCT CTCTCTCTCT CTCTCAAAAA 180

CACTTGGTCT GTTATTTTTC GAAATTGTCA GTCATAGTTA TCTGTTAGAC CAAAGCTGGT 240

AAGACATTTA TTACATTGCC TCCTACAACT TCATCAGCTA ATGTATTTGC TATATAGCAA 300

TTACATATGG ATATATTATC TTAGGGGATG GCCAGTATAA AACTGTCACT GAGGAAAGGA 360

(2) INFORMATION FOR SEQ ID NO:15:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 288 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc = "SYNTHETIC DNA"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:15:

CCTCCCACCT AGCCTCCCCA GTAGCTAGGA CTATAGGCGT GCCCACCAAG CTCAGCTATT 60

TTTTATTTAG TAGAGACGGG GTTTCGGCAG CTTAGGCCTC GTTCGAACTC CAGTGTGTGT 120

GTGTGTGTGT GTGTGTGTGT GTGTGTGTGT GTGTGTGTGT GTGTAGATAT TTATTCCCCC 180

TCCCCCTTGG AAAAGTAAGT AAGCTCCTAC TAGGAATTTA AAACCTGCTT GATCTATATA 240

AAGACAAACA AGGAAAGACA AACATGGGGG CAGGAAGGAA GGCAGATC 288

(2) INFORMATION FOR SEQ ID NO:16:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 141 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc = "SYNTHETIC DNA"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:16:

TCGAGGTAGA TTTGTATTAT ATCCCATGTA CACACACACA CACACACACA CACACACACA 60

CACACACAGA CTTAATCTGT TTACAGAAAT AAAAGGAATA AAATACCGTT TCTACTATAC 120

ACCAAAACTA GCCATCTTGA C 141

(2) INFORMATION FOR SEQ ID NO:17:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 305 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc = "SYNTHETIC DNA"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:17:

CCCTGAGAAG GCTTCCTCCT GAGTATGCAT AAACATTCAC AGCTTGCATG CGTGTGTGTG 60

TGTGTGTGTG TGTGTATGTT TGCTTGCACT GTAAAAACAA TTGCAACATC AACAGAAATA 120

AAAATTAAAG GAATAATTCT CCTCCGACTC TGCCGTTCCA TCCAGTGAAA CTCTTCATTC 180

TGGGGTAAAG TTCCTTCAGT TCTTTCATAG ATAGGTATAT ACTTCATAAG TCAAACAATC 240

AGGCTGGGTG CAGTAGCTCA TGCCTGTAAT CCCAGCCCTT TGGGAGGCCG AGCTGGGCAG 300

ATCGA 305

(2) INFORMATION FOR SEQ ID NO:18:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 341 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc = "SYNTHETIC DNA"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:18:

TCCACCCGCC TTGGCCTCCC AAAGCCTGGG ATTACAGGCG TGACTGCCGC ACCCAGCTGT 60

AAACTGGTTT AATGGTAGAT TTTAGGTATT AACAATAGAT AAAAAGATAC TTTTGGCATA 120

CTGTGTATTG GGATGGGGTT AGAACAGGTG TCTACCCAAG ACATTTACTT AAAATCGCCC 180

TCGAAATGCT ATGTGAGCTG TGTGTGTGTG TGTGTGTGTG TGTGTATTAA GGAAAAGCAT 240

GAAAGTATTT ATGCTTGATT TTTTTTTTTA CTCATAGCTT CATAGTGGAC AGATACATAG 300

TCTAAATCAA AATGTTTAAA CTTTTTATGT CACTTGCTGT C 341

(2) INFORMATION FOR SEQ ID NO:19:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 278 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:19:

Met Ala Met Ser Ser Gly Gly Ser Gly Gly Gly Val Pro Glu Gln Glu
1   5   10   15

Asp Ser Val Leu Phe Arg Arg Gly Thr Gly Gln Ser Asp Asp Ser Asp
            20   25   30

Ile Trp Asp Asp Thr Ala Leu Ile Lys Ala Tyr Asp Lys Ala Val Ala
        35   40   45

Ser Phe Lys His Ala Leu Lys Asn Gly Asp Ile Cys Glu Thr Ser Gly
    50   55   60

Lys Pro Lys Thr Thr Pro Lys Arg Lys Pro Ala Lys Lys Asn Lys Ser
65   70   75   80

Gln Lys Lys Asn Thr Ala Ala Ser Leu Gln Gln Trp Lys Val Gly Asp
                85   90   95

Lys Cys Ser Ala Ile Trp Ser Glu Asp Gly Cys Ile Tyr Pro Ala Thr
            100   105   110

Ile Ala Ser Ile Asp Phe Lys Arg Glu Thr Cys Val Val Val Tyr Thr
        115   120   125

```
Gly Tyr Gly Asn Arg Glu Glu Gln Asn Leu Ser Asp Leu Leu Ser Pro
    130                 135                 140

Ile Cys Glu Val Ala Asn Asn Ile Glu Gln Asn Ala Gln Glu Asn Glu
145                 150                 155                 160

Asn Glu Ser Gln Val Ser Thr Asp Glu Ser Glu Asn Ser Arg Ser Pro
                165                 170                 175

Gly Asn Lys Ser Asp Asn Ile Lys Pro Lys Ser Ala Pro Trp Asn Ser
            180                 185                 190

Phe Leu Pro Pro Pro Pro Pro Met Pro Gly Pro Arg Leu Gly Pro Gly
        195                 200                 205

Lys Pro Gly Leu Lys Phe Asn Gly Pro Pro Pro Pro Pro Pro Pro Pro
    210                 215                 220

Pro Pro His Leu Leu Ser Cys Trp Leu Pro Pro Phe Pro Ser Gly Pro
225                 230                 235                 240

Pro Ile Ile Pro Pro Pro Pro Pro Ile Cys Pro Asp Ser Leu Asp Asp
                245                 250                 255

Ala Asp Ala Leu Gly Ser Met Leu Ile Ser Trp Tyr Met Ser Gly Tyr
            260                 265                 270

His Thr Gly Tyr Tyr Met
        275
```

(2) INFORMATION FOR SEQ ID NO:20:

(i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 885 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(ix) FEATURE:
- (A) NAME/KEY: CDS
- (B) LOCATION: 18..881

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:20:

```
CGGCGTGGTA GCAGGCC ATG GCG ATG GGC AGT GGC GGA GCG GGC TCC GAG        50
                   Met Ala Met Gly Ser Gly Gly Ala Gly Ser Glu
                     1               5                  10
```

CAG GAA GAT ACG GTG CTG TTC CGG CGT GGC ACC GGC CAG AGT GAT GAT 98
Gln Glu Asp Thr Val Leu Phe Arg Arg Gly Thr Gly Gln Ser Asp Asp
15 20 25

TCT GAC ATT TGG GAT GAT ACA GCA TTG ATA AAA GCT TAT GAT AAA GCT 146
Ser Asp Ile Trp Asp Asp Thr Ala Leu Ile Lys Ala Tyr Asp Lys Ala
30 35 40

GTG GCT TCC TTT AAG CAT GCT CTA AAG AAC GGT GAC ATT TGT GAA ACT 194
Val Ala Ser Phe Lys His Ala Leu Lys Asn Gly Asp Ile Cys Glu Thr
45 50 55

CCA GAT AAG CCA AAA GGC ACA GCC AGA AGA AAA CCT GCC AAG AAG AAT 242
Pro Asp Lys Pro Lys Gly Thr Ala Arg Arg Lys Pro Ala Lys Lys Asn
60 65 70 75

AAA AGC CAA AAG AAG AAT GCC ACA ACT CCC TTG AAA CAG TGG AAA GTT 290
Lys Ser Gln Lys Lys Asn Ala Thr Thr Pro Leu Lys Gln Trp Lys Val
80 85 90

GGT GAC AAG TGT TCT GCT GTT TGG TCA GAA GAC GGC TGC ATT TAC CCA 338
Gly Asp Lys Cys Ser Ala Val Trp Ser Glu Asp Gly Cys Ile Tyr Pro
95 100 105

GCT ACT ATT ACG TCC ATT GAC TTT AAG AGA GAA ACC TGT GTC GTG GTT 386
Ala Thr Ile Thr Ser Ile Asp Phe Lys Arg Glu Thr Cys Val Val Val
110 115 120

TAT ACT GGA TAT GGA AAC AGA GAG GAG CAA AAC TTA TCT GAC CTA CTT 434
Tyr Thr Gly Tyr Gly Asn Arg Glu Glu Gln Asn Leu Ser Asp Leu Leu
125 130 135

TCC CCG ACC TGT GAA GTA GCT AAT AGT ACA GAA CAG AAC ACT CAG GAG 482
Ser Pro Thr Cys Glu Val Ala Asn Ser Thr Glu Gln Asn Thr Gln Glu
140 145 150 155

AAT GAA AGT CAA GTT TCC ACA GAC GAC AGT GAA CAC TCC TCC AGA TCG 530
Asn Glu Ser Gln Val Ser Thr Asp Asp Ser Glu His Ser Ser Arg Ser
160 165 170

CTC AGA AGT AAA GCA CAC AGC AAG TCC AAA GCT GCT CCG TGG ACC TCA 578
Leu Arg Ser Lys Ala His Ser Lys Ser Lys Ala Ala Pro Trp Thr Ser
175 180 185

TTT CTT CCT CCA CCA CCC CCA ATG CCA GGG TCA GGA TTA GGA CCA GGA 626
Phe Leu Pro Pro Pro Pro Pro Met Pro Gly Ser Gly Leu Gly Pro Gly
190 195 200

AAG CCA GGT CTA AAA TTC AAC GGC CCG CCG CCG CCG CCT CCA CTA CCC 674
Lys Pro Gly Leu Lys Phe Asn Gly Pro Pro Pro Pro Pro Pro Leu Pro
205 210 215

CCT CCC CCC TTC CTG CCG TGC TGG ATG CCC CCG TTC CCT TCA GGA CCA 722
Pro Pro Pro Phe Leu Pro Cys Trp Met Pro Pro Phe Pro Ser Gly Pro
220 225 230 235

CCA ATA ATC CCG CCA CCC CCT CCC ATC TCT CCC GAC TGT CTG GAT GAC 770
Pro Ile Ile Pro Pro Pro Pro Pro Ile Ser Pro Asp Cys Leu Asp Asp
240 245 250

ACT GAT GCC CTG GGC AGT ATG CTA ATC TCT TGG TAC ATG AGT GGC TAC 818
Thr Asp Ala Leu Gly Ser Met Leu Ile Ser Trp Tyr Met Ser Gly Tyr
255 260 265

CAC ACT GGC TAC TAT ATG GGT TTC AGA CAA AAT AAA AAA GAA GGA AAG 866
His Thr Gly Tyr Tyr Met Gly Phe Arg Gln Asn Lys Lys Glu Gly Lys
270 275 280

TGC TCA CAT ACA AAT TAAG 885
Cys Ser His Thr Asn
285

(2) INFORMATION FOR SEQ ID NO:21:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 288 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:21:

Met Ala Met Gly Ser Gly Gly Ala Gly Ser Glu Gln Glu Asp Thr Val
1 5 10 15

Leu Phe Arg Arg Gly Thr Gly Gln Ser Asp Asp Ser Asp Ile Trp Asp
20 25 30

Asp Thr Ala Leu Ile Lys Ala Tyr Asp Lys Ala Val Ala Ser Phe Lys
35 40 45

His Ala Leu Lys Asn Gly Asp Ile Cys Glu Thr Pro Asp Lys Pro Lys
50 55 60

Gly Thr Ala Arg Arg Lys Pro Ala Lys Lys Asn Lys Ser Gln Lys Lys
65 70 75 80

Asn Ala Thr Thr Pro Leu Lys Gln Trp Lys Val Gly Asp Lys Cys Ser
85 90 95

Ala Val Trp Ser Glu Asp Gly Cys Ile Tyr Pro Ala Thr Ile Thr Ser
100 105 110

```
Ile Asp Phe Lys Arg Glu Thr Cys Val Val Val Tyr Thr Gly Tyr Gly
        115                 120                 125

Asn Arg Glu Glu Gln Asn Leu Ser Asp Leu Leu Ser Pro Thr Cys Glu
    130                 135                 140

Val Ala Asn Ser Thr Glu Gln Asn Thr Gln Glu Asn Glu Ser Gln Val
145                 150                 155                 160

Ser Thr Asp Asp Ser Glu His Ser Ser Arg Ser Leu Arg Ser Lys Ala
                165                 170                 175

His Ser Lys Ser Lys Ala Ala Pro Trp Thr Ser Phe Leu Pro Pro Pro
            180                 185                 190

Pro Pro Met Pro Gly Ser Gly Leu Gly Pro Gly Lys Pro Gly Leu Lys
        195                 200                 205

Phe Asn Gly Pro Pro Pro Pro Pro Pro Leu Pro Pro Pro Pro Phe Leu
    210                 215                 220

Pro Cys Trp Met Pro Pro Phe Pro Ser Gly Pro Pro Ile Ile Pro Pro
225                 230                 235                 240

Pro Pro Pro Ile Ser Pro Asp Cys Leu Asp Asp Thr Asp Ala Leu Gly
                245                 250                 255

Ser Met Leu Ile Ser Trp Tyr Met Ser Gly Tyr His Thr Gly Tyr Tyr
            260                 265                 270

Met Gly Phe Arg Gln Asn Lys Lys Glu Gly Lys Cys Ser His Thr Asn
        275                 280                 285
```

(2) INFORMATION FOR SEQ ID NO:22:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 3246 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:22:

```
CCTCCCGGGC ACCGTACTGT TCCGCTCCCA GAAGCCCCGG GCGCCGGAAG TCGTCACTCT     60

TAAGAAGGGA CGGGGCCCCA CGCTGCGCAC CCGCGGGTTT GCTATGGCGA TGAGCAGCGG    120
```

CGGCAGTGGT GGCGGCGTCC CGGAGCAGGA GGATTCCGTG CTGTTCCGGC GCGGCACAGG 180

CAGGTGAGGT CGCAGCCAGT GCAGTCTCCC TATTAGCGCT CTCAGCACCC TTCTTCCGGC 240

CCAACTCTCC TTCCGCAGTA ATTTTGTTAT GTGTGGATTA AGATGACTCT TGGTACTAAC 300

ATACATTTTC TGATTAAACC TATCTGACAT GAGTTGTTTT TATTTCTTAC CCTTTCCAGA 360

GCGATGATTC TGACATTTGG GATGATACAG CACTGATAAA AGCATATGAT AAAGCTGTGG 420

CTTCATTTAA GGTATGAAAT GCTTGTTAGT CGTTTTCTTA TTTTCTCGTT ATTCATTTGG 480

AAAGGAATTG ATAACATACG ATAAAGTGTT AAGTGCTTTC TGAGGTGACG GAGCCTTGAG 540

ACTAGCTTAT AGTAGTAACT GGGTTATGTC GTGACTTTTA TTCTGTGCAC CACCCTGTAA 600

CATGTACATT TTTATTCCTA TTTTCGTAGC ATGCTCTAAA GAATGGTGAC ATTTGTGAAA 660

CTTGGGGTAA ACCAAAAACC ACACCTAAAA GAAAACCTGC TAAGAAGAAT AAAAGCCAAA 720

AGAAGAATAC TGCAGCTTCC TTACAACAGG TTATTTTAAA ATGTTGAGGA TTTAACTTCA 780

AAGGATGTCT CATTAGTCCT TATTTAATAG TGTAAAATGT CTTTAACTGC AGGTCGATCA 840

AAACGAGATG ATAGTTTGCC CTCTTCAAAA GAAATGTGTG CATGTATATA TCTTTGATTT 900

CTTTTGTAGT GGAAAGTTGG GGACAAATGT TCTGCCATTT GGTCAGAAGA CGGTTGCATT 960

TACCCAGCTA CCATTGCTTC AATTGATTTT AAGAGAGAAA CCTGTGTTGT GGTTTACACT 1020

GGATATGGAA ATAGAGAGGA GCAAAATCTG TCCGATCTAC TTTCCCCAAT CTGTGAAGTA 1080

GCTAATAATA TAGAACAGAA TGCTCAAGAG GTAAGGATAC AAAAAAAAAA AAATTCAATT 1140

TCTGGAAGCA GAGACTAGAT GAGAAACTGT TAAACAGTAT ACAACCGAGG CATTAATTTT 1200

TTCTTAATCA CACCCTTATA ACAAAAACCT GCATATTTTT TCTTTTTAAA GAATGAAAAT 1260

GAAAGCCAAG TTTCAACAGA TGAAAGTGAG AACTCCAGGT CTCCTGGAAA TAAATCAGAT 1320

AACATCAAGC CCAAATCTGC TCCATGGAAC TCTTTTCTCC CTCCACCACC CCCCATGCCA 1380

GGGCCAAGAC TGGGACCAGG AAAGGTAAAC CTTCTATGAA AGTTTTCCAG AAAATAGTTA 1440

ATGTCGGGAC ATTTAACCTC TCTGTTAACT AATTTGTAGC TCTCCAATAT TCTGGGTAAT 1500

TATTTTTATC CTTTTGGTTT TGAGTCCTTT TTATTCCTAT CATATTGAAA TTGGTAAGTT 1560

AATTTTCCTT TGAAATATTC CTTATAGCCA GGTCTAAAAT TCAATGGCCC ACCACCGCCA 1620

CCGCCACCAC CACCACCCCA CTTACTATCA TGCTGGCTGC CTCCATTTCC TTCTGGACCA 1680

CCAGTAAGTA AAAAAGAGTA TAGGTTAGAT TTTGCTTTCA CATACAATTT GATAATAGAC 1740
TTTACTTTTT GTTTACTGGA TATAAACAAT ATCTTTTTCT GTCTCCAGAT AATTCCCCCA 1800
CCACCTCCCA TATGTCCAGA TTCTCTTGAT GATGCTGATG CTTTGGGAAG TATGTTAATT 1860
TCATGGTACA TGAGTGGCTA TCATACTGGC TATTATATGG TAAGTAATCA CTCAGCATCT 1920
TTTCCTGACA ATTTTTTTGT AGTTATGTGA CTTTGTTTGG TAAATTTATA AAATACTACT 1980
CTGCAGCCTA ATAATTGTTT TCTTTGGGAT AACTTTTAAA GTACATTAAA AGACTATCAA 2040
CTTAATTTCT GATCATATTT TGTTGAATAA AATAAGTAAA ATGTCTTGTG AAACAAAATG 2100
CTTTTTAACA TCCATATAAA GCTATCTATA TATAGCTATC TATGTCTATA TAGCTATTTT 2160
TTTTAACTTC CTTTTATTTT CCTTACAGGG TTTCAGACAA AATCAAAAAG AAGGAAGGTG 2220
CTCACATTCC TTAAATTAAG GAGTAAGTCT GCCAGCATTA TGAAAGTGAA TCTTACTTTT 2280
GTAAAACTTT ATGGTTTGTG GAAAACAAAT GTTTTTGAAC AGTTAAAAAG TTCAGATGTT 2340
AAAAAGTTGA AAGGTTAATG TAAAACAATC AATATTAAAG AATTTTGATG CCAAAACTAT 2400
TAGATAAAAG GTTAATCTAC ATCCCTACTA GAATTCTCAT ACTTAACTGG TTGGTTATGT 2460
GGAAGAAACA TACTTTCACA ATAAAGAGCT TTAGGATATG ATGCCATTTT ATATCACTAG 2520
TAGGCAGACC AGCAGACTTT TTTTTATTGT GATATGGGAT AACCTAGGCA TACTGCACTG 2580
TACACTCTGA CATATGAAGT GCTCTAGTCA AGTTTAACTG GTGTCCACAG AGGACATGGT 2640
TTAACTGGAA TTCGTCAAGC CTCTGGTTCT AATTTCTCAT TTGCAGGAAA TGCTGGCATA 2700
GAGCAGCACT AAATGACACC ACTAAAGAAA CGATCAGACA GATCTGGAAT GTGAAGCGTT 2760
ATAGAAGATA ACTGGCCTCA TTTCTTCAAA ATATCAAGTG TTGGGAAAGA AAAAAGGAAG 2820
TGGAATGGGT AACTCTTCTT GATTAAAAGT TATGTAATAA CCAAATGCAA TGTGAAATAT 2880
TTTACTGGAC TCTTTTGAAA AACCATCTAG TAAAAGACTG GGGTGGGGGT GGGAGGCCAG 2940
CACGGTGGTG AGGCAGTTGA GAAAATTTGA ATGTGGATTA GATTTTGAAT GATATTGGAT 3000
AATTATTGGT AATTTTATGG CCTGTGAGAA GGGTGTTGTA GTTTATAAAA GACTGTCTTA 3060
ATTTGCATAC TTAAGCATTT AGGAATGAAG TGTTAGAGTG TCTTAAAATG TTTCAAATGG 3120
TTTAACAAAA TGTATGTGAG GCGTATGTGG CAAAATGTTA CAGAATCTAA CTGGTGGACA 3180
TGGCTGTTCA TTGTACTGTT TTTTTCTATC TTCTATATGT TTAAAAGTAT ATAATAAAAA 3240

TATTTA 3246

(2) INFORMATION FOR SEQ ID NO:23:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 637 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:23:

```
GATCTGCCTT CCTTCCTGCC CCCATGTTTG TCTTTCCTTG TTTGTCTTTA TATAGATCAA    60
GCAGGTTTTA AATTCCTAGT AGGAGCTTAC ATTTACTTTT CCAAGGGGGA GGGGGAATAA   120
ATATCTACAC ACACACACAC ACACACACCA CACTGGAGTT CGAGACGAGG CCTAAGCAAC   180
ATGCCGAAAC CCCGTCTCTA CTAAATACAA AAAATAGCTG AGCTTGGTGG CGCACGCCTA   240
TAGTCCTAGC TACTGGGGAG GCTGAGGTGG GAGGATCGCT TGAGCCCAAG AAGTCGAGGC   300
TGCAGTGAGC CGAGATCGCG CCGCTGCACT CCAGCCTGAG CGACAGGGCG AGGCTCTGTC   360
TCAAAACAAA CAAACAAAAA AAAAAAGGAA AGGAAATATA ACACAGTGAA ATGAAAGGAT   420
TGAGAGAAAT GAAAAATATA CACGCCACAA ATGTGGGAGG GCGATAACCA CTCGTAGAAA   480
GCGTGAGAAG TTACTACAAG CGGTCCTCCC GGGCACCGTA CTGTTCCGCT CCCAGAAGCC   540
CCGGGCGCCG GAAGTCGTCA CTCTTAAGAA GGGACGGGGC CCCACGCTGC GCACCCGCGG   600
GTTTGCTATG GCGATGAGCA GCGGCGGCAG TGGTGGC                            637
```

(2) INFORMATION FOR SEQ ID NO:24:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 19 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid

(iii) HYPOTHETICAL: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:24:

AGGGCGAGGC TCTGTCTCA 19

(2) INFORMATION FOR SEQ ID NO:25:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 19 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid

(iii) HYPOTHETICAL: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:25:

CGGGAGGACC GCTTGTAGT 19

(2) INFORMATION FOR SEQ ID NO:26:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 19 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid

(iii) HYPOTHETICAL: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:26:

GCCGGAAGTC GTCACTCTT 19

(2) INFORMATION FOR SEQ ID NO:27:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid

(iii) HYPOTHETICAL: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:27:

GGGTGCTGAG AGCGCTAATA 20

(2) INFORMATION FOR SEQ ID NO:28:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid

(iii) HYPOTHETICAL: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:28:

TGTGTGGATT AAGATGACTC 20

(2) INFORMATION FOR SEQ ID NO:29:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 19 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid

(iii) HYPOTHETICAL: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:29:

CACTTTATCG TATGTTATC 19

(2) INFORMATION FOR SEQ ID NO:30:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 22 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid

(iii) HYPOTHETICAL: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:30:

CTGTGCACCA CCCTGTAACA TG 22

(2) INFORMATION FOR SEQ ID NO:31:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 19 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid

(iii) HYPOTHETICAL: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:31:

AAGGACTAAT GAGACATCC 19

(2) INFORMATION FOR SEQ ID NO:32:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid

(iii) HYPOTHETICAL: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:32:

CGAGATGATA GTTTGCCCTC 20

(2) INFORMATION FOR SEQ ID NO:33:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 24 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid

(iii) HYPOTHETICAL: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:33:

AGCTACTTCA CAGATTGGGG AAAG 24

(2) INFORMATION FOR SEQ ID NO:34:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid

(iii) HYPOTHETICAL: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:34:

CTCATCTAGT CTCTGCTTCC 20

(2) INFORMATION FOR SEQ ID NO:35:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 24 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid

(iii) HYPOTHETICAL: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:35:

TGGATATGGA AATAGAGAGG GAGC 24

(2) INFORMATION FOR SEQ ID NO:36:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 22 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid

(iii) HYPOTHETICAL: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:36:

CACCCTTATA ACAAAAACCT GC 22

(2) INFORMATION FOR SEQ ID NO:37:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 23 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid

(iii) HYPOTHETICAL: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:37:

GAGAAAGGAG TTCCATGGAG CAG 23

(2) INFORMATION FOR SEQ ID NO:38:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid

(iii) HYPOTHETICAL: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:38:

GAGAGGTTAA ATGTCCCGAC 20

(2) INFORMATION FOR SEQ ID NO:39:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 22 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid

(iii) HYPOTHETICAL: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:39:

GTGAGAACTC CAGGTCTCCT GG 22

(2) INFORMATION FOR SEQ ID NO:40:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid

(iii) HYPOTHETICAL: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:40:

TGAGTCTGTT TGACTTCAGG 20

(2) INFORMATION FOR SEQ ID NO:41:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 22 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid

(iii) HYPOTHETICAL: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:41:

GAAGGAAATG GAGGCAGCCA GC 22

(2) INFORMATION FOR SEQ ID NO:42:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid

(iii) HYPOTHETICAL: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:42:

TTTCTACCCA TTAGAATCTG G 21

(2) INFORMATION FOR SEQ ID NO:43:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 24 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid

(iii) HYPOTHETICAL: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:43:

CCCCACTTAC TATCATGCTG GCTG 24

(2) INFORMATION FOR SEQ ID NO:44:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 24 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid

(iii) HYPOTHETICAL: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:44:

CCAGACTTTA CTTTTTGTTT ACTG 24

(2) INFORMATION FOR SEQ ID NO:45:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid

(iii) HYPOTHETICAL: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:45:

ATAGCCACTC ATGTACCATG A 21

(2) INFORMATION FOR SEQ ID NO:46:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 24 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid

(iii) HYPOTHETICAL: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:46:

AAGAGTAATT TAAGCCTCAG ACAG 24

(2) INFORMATION FOR SEQ ID NO:47:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 24 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid

(iii) HYPOTHETICAL: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:47:

CTCCCATATG TCCAGATTCT CTTG 24

(2) INFORMATION FOR SEQ ID NO:48:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 25 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid

(iii) HYPOTHETICAL: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:48:

AGACTATCAA CTTAATTTCT GATCA 25

(2) INFORMATION FOR SEQ ID NO:49:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 24 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid

(iii) HYPOTHETICAL: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:49:

TAAGGAATGT GAGCACCTTC CTTC 24

(2) INFORMATION FOR SEQ ID NO:50:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 25 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid

(iii) HYPOTHETICAL: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:50:

AGACTATCAA CTTAATTTCT GATCA 25

(2) INFORMATION FOR SEQ ID NO:51:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 24 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid

(iii) HYPOTHETICAL: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:51:

GTAAGATTCA CTTTCATAAT GCTG 24

(2) INFORMATION FOR SEQ ID NO:52:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 21 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid

(iii) HYPOTHETICAL: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:52:

CTTTATGGTT TGTGGAAAAC A 21

(2) INFORMATION FOR SEQ ID NO:53:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid

(iii) HYPOTHETICAL: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:53:

GGCATCATAT CCTAAAGCTC 20

(2) INFORMATION FOR SEQ ID NO:54:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 23 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid

(iii) HYPOTHETICAL: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:54:

GTAATAACCA AATGCAATGT GAA 23

(2) INFORMATION FOR SEQ ID NO:55:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid

(iii) HYPOTHETICAL: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:55:

CTACAACACC CTTCTCACAG 20

(2) INFORMATION FOR SEQ ID NO:56:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid

(iii) HYPOTHETICAL: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:56:

GGTGTCCACA GAGGACATGG 20

(2) INFORMATION FOR SEQ ID NO:57:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 24 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid

(iii) HYPOTHETICAL: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:57:

AAGAGTTAAC CCATTCCAGC TTCC 24

Survival motor neuron (SMN) gene: a gene for spinal muscular atrophy

BACKGROUND OF THE INVENTION

1. Field of the Invention

The present invention relates to the discovery of the human survival motor-neuron gene or SMN gene which is a chromosome 5-SMA (Spinal Muscular Atrophy) determining gene. The present invention further relates to the nucleotide sequence encoding the SMN gene and corresponding amino acid sequence, a vector containing the gene encoding the SMN protein or a DNA sequence corresponding to the gene and transformant strains containing the SMN gene or a DNA sequence corresponding to the gene.

More particularly, the present invention relates to means and methods for detecting motor neuron diseases having symptoms of muscular weakness with or without sensory changes such as amytrophic lateral sclerosis (ALS), spinal muscular atrophy (SMA), primary lateral sclerosis (PLS), arthrogryposis multiplex congenita (AMC), and the like. The methods for detecting such motor neuron diseases include, but are not limited to, the use of specific DNA primers in the PCR technique, the use of hybridization probes and the use of polyclonal and monoclonal antibodies.

Even more particularly, the present invention relates to the use of the human SMN gene or part of the gene, cDNA, oligonucleotide or the encoded protein or part thereof in therapy by insertion of the human SMN gene or part of the gene, cDNA, oligonucleotide or the encoded protein or part thereof, if required, into engineered viruses or vectors that serve as harmless carriers to transport the gene or part of the gene, cDNA, oligonucleotide or the encoded protein or part thereof to the body's cells including bone marrow cells.

The invention further relates to antigen sequences directed to the SMN gene.

In order to provide means for the therapy of motor neuron diseases, the invention also relates to the protein encoded by the SMN gene.

The present invention also relates to the isolation of the mouse SMN gene, the nucleotide sequence encoding the mouse SMN gene and corresponding amino acid sequence. A transgenic mouse model that hyperexpresses all or part of the SMN gene and a transgenic mouse model produced by homologous recombination with a mutated SMN gene is also described.

2. State of the Art

Degenerative motor neuron diseases can be placed into three major categories. Amyotrophic lateral sclerosis or ALS, motor neuron diseases such as spinal muscular atrophy (SMA) and motor neuron diseases associated with other degenerative disorders such as primary lateral sclerosis (PLS).

Amyotrophic lateral sclerosis (ALS) is the most frequently encountered form of progressive neuron disease and is characteristically a disorder of middle age. The disease is characterized by progressive loss of motor neurons, both in the cerebral cortex and in the anterior horns of the spinal cord, together with their homologues in some motor nuclei of the brainstem. It typically affects both upper and lower motor neurons, although variants may predominantly involve only particularly subsets of motor neurons, particularly early in the course of illness.

ALS is evidenced by the development of asymmetric weakness, with fatigue and cramping of affected muscles. The weakness is accompanied by visible wasting and atrophy of the muscles evolves and over time, more and

more muscles become involved until the disorder takes on a symmetric distribution in all regions, including muscles of chewing, swallowing and movement of the face and tongue. Fifty percent of patients having ALS can be expected to die within three to five years from the onset of the disease. Presently, there is no treatment that has influence on the pathologic process of ALS.

Spinal muscular atrophies (SMA) are characterized by degeneration of anterior horn cells of the spinal cord leading to progressive symmetrical limb and trunk paralysis associated with muscular atrophy. SMA represents the second most common fatal, autosomal recessive disorder after cystic fibrosis (1 out 6000 newborns). Childhood SMA is classically subdivided into three clinical groups on the basis of age of onset and clinical course. The acute form of Werdnig-Hoffmann disease (Type I) is characterized by severe generalized muscle weakness and hypotonia at birth or in the 3 months following birth. Death, from respiratory failure, usually occurs within the first two years. This disease may be distinguished from the intermediate (Type II) and juvenile (Type III, Kugelberg-Welander disease) forms. Type II children were able to sit but unable to stand or walk unaided, and they live beyond 4 years. Type III patients had proximal muscle weakness, starting after the age of two. The underlying biochemical defect remains unknown. In addition there is known to exist a slowly evolving adult form of SMA, sometimes referred to as SMA IV.

Primary lateral slcerosis (PLS) is a variant of ALS and occurs as a sporadic disease of late life. Neuropathologically in PLS there is a degeneration of the corticospinal (pyramidal) tracts, which appear almost normal at brainstem levels but become increasingly atrophic as they descend through the spinal column. The lower limbs are affected earliest and most severely.

Arthrogryposis Multiplex Congenita (AMC) is a frequent syndrome characterized by congenital joint fixation (incidence of 1 out of 3000 live births) resulting from decreased fetal movements *in utero* (Stern, W.G., JAMA, 81:1507-

1510 (1923) ; Hall, J.G., Clin. Orthop., 194:44-53 (1985)). AMC has been ascribed to either oligo-hydramnios or a variety of diseases involving the central nervous system, skeletal muscle, or spinal cord. Since neuronal degeneration and neuronophagia occur in the anterior horns, it has been hypothesized that the AMC of neurogenic origin could be related to acute spinal muscular atrophy; SMA Type I Werdnig-Hoffman disease (Banker, B.Q., Hum. Pathol., (1986); 117:656-672).

The detection and clinical diagnosis for ALS, AMC, SMA and PLS is quite limited to muscle biopsies, the clinical diagnosis by a physician and electromyography (EMG). For example, the clinical criteria for diagnosing SMA is set forth in the Clinical Criteria International SMA Consortium (Munsat T.L., Neuromuscular Disorders, Vol. 1, p. 81 (1991)). But due to the complications of the various tests to detect motor neuron disorders, the clinician usually attempts to eliminate various categories of other disease states such as structural lesions, infections, intoxications, metabolic disorders and heriditary biochemical disorders prior to utilizing the above-described test methods.

Presently there is no treatment for any of the above-mentioned motor neuron disorders. Basic rehabilitative measures, including mechanical aids of various kinds, may help patients that have these diseases overcome the effects of their disabilities, but often confining respiratory support systems are necessary to have the patient survive longer.

Accordingly, it is an object of the present invention to characterize the SMA gene which is responsible for SMA disorders and to clone the SMA gene into a vector, for example a plasmid, a cosmid, a phage, a YAC vector, that can be used in the transformation process to produce large quantities of the SMN gene and SMN protein.

In yet another aspect of the invention is the use of primers and hybridization probes to detect and diagnose patients having motor neuron

disorders such as AMC, ALS, SMA and PLS. Yet another aspect of the present invention is the use of the SMN gene or part thereof or cDNA, oligonucleotides, protein or part thereof in therapy to correct disorders present in, for example AMC, SMA, ALS and PLS patients, especially gene disorders.

In yet another aspect, the present invention provides monoclonal and polyclonal antibodies for detection of SMN gene defects in SMA patients.

Another object of the present invention provides the characterization of the SMA gene in the mouse. A transgenic mouse model is presented that hyperexpresses all or part of the SMN gene or a transgenic mouse that by homologous recombination with a mutated mouse SMN gene produces abnormalities in the SMN gene is also described.

According to a further aspect of the invention, the therapy of motor neuron diseases can involve the protein encoded by the SMN gene.

These and other objects are achieved by the present invention as evidenced by the summary of the invention, the description of the preferred embodiments and the claims.

OBJECTS AND SUMMARY OF THE INVENTION

It is an object of the present invention to provide a novel human Survival Motor Neuron gene or SMN gene, its DNA sequence and amino acid sequence.

Another aspect of the present invention provides a novel mouse Survival Motor Neuron gene or SMN gene, its DNA sequence and amino acid sequence.

Yet another aspect of the present invention is the provision of a vector which is capable of replicating in a host microorganism to provide large quantities of the human or mouse SMN protein.

Yet another aspect of the present invention is the provision of specific DNA sequences that can be used to detect and diagnose spinal muscular atrophy and other motor neuron disorders. These DNA sequences can be used as primers in the polymerase chain reaction to amplify and detect the SMN gene sequence, a truncated or mutated version of the SMN gene sequence or lack of said sequence which leads to the diagnosis of SMA, AMC, and other motor neuron disorders.

Yet another aspect of the present invention provides a transgenic mouse that hyperexpresses all or part of the SMN gene or a transgenic mouse that by homologous recombination with a mutated mouse SMN gene produces abnormalities in the SMN gene is also described.

The inventors have identified two genes respectively designated T-BCD541 and C-BCD541, which are involved in motor neuron disorders.

The T-BCD541 gene is responsible for the motor neuron diseases of the SMA type, since its alteration either by partial or total deletion, by mutation or any other modification, is sufficient to lead to a pathological state at the clinical electromyographic or muscle morphological levels.

The C-BCD541 gene is different from the T-BCD541 gene, at the level of the cDNA, since two nucleotides are modified. This C-BCD541 gene is nevertheless not correctly processed during the transcription in controls and patients suffering from motor neuron diseases. The genomic DNA of the C-BCD541 gene is not correctly spliced during the transcription providing thus for an abnormal transcript. The difference between the splicing of the T-BCD541

and the C-BCD541 gene results from differences in the sequence of the introns of these genes.

The present invention thus further characterizes the structure and organization of the human SMN gene which was found to be approximately 20 kb in length and consists of 9 exons interrupted by 8 introns. The nucleotide sequence, amino acid sequence as well as the exon-intron boundaries of the human SMN gene is set forth in Figure 10. All exon-intron boundaries display the consensus sequence found in other human genes. A polyadenylation consensus site is localized about 550 bp downstream from the stop codon (Figure 10). The entire intron/exon structure of the SMN gene permits the characterizations of the SMN gene mutations in SMA disease or other motor neuron diseases.

The present invention also defines means for the detection of genomic abnormalities relating to motor neuron diseases at the level of the T-BCD541 gene or at the level of the C-BCD541 gene.

The genes of the invention can be further defined in that each of them comprise intronic sequences corresponding to the following sequences :

In the T-BCD541 gene

- for intron n° 6 :

5' AATTTTTAAATTTTTTGTAGAGACAGGGTCTCATTATGTTGCCCAGGGTG
GTGTCAAGCTCCAGGTCTCAAGTGATCCCCCTACCTCCGCCTCCCAAAGTTGT
GGGATTGTAGGCATGAGCCACTGCAAGAAAACCTTAACTGCAGCCTAATAATT
GTTTTCTTTGGGATAACTTTTAAAGTACATTAAAAGACTATCAACTTAATTTC
TGATCATATTTTGTTGAATAAAATAAGTAAAATGTCTTGTGAACAAAATGCTT
TTTAACATCCATATAAAGCTATCTATATATAGCTATCTATGTCTATATAGCTA
TTTTTTTTAACTTCCTTTTATTTTCCTTACAG 3'

- for intron n° 7 :

5' GTAAGTCTGCCAGCATTATGAAAGTGAATCTTACTTTTGTAAAACTTTAT
GCTTTGTGGAAAACAAATGTTTTTGAACAGTTAAAAAGTTCAGATGTTAAAAA
GTTGAAAGGTTAATGTAAAACAATCAATATTAAAGAATTTTGATGCCAAAACT
ATTAGATAAAAGGTTAATCTACATCCCTACTAGAATTCTCATACTTAACTGGT
TGGTTATGTGGAAGAAACATACTTTCACAATAAAGAGCTTTAGGATATGATGC
CATTTTATATCACTAGTAGGCAGACCAGCAGACTTTTTTTATTGTGATATGG
GATAACCTAGGCATACTGCACTGTACACTCTGACATATGAAGTGCTCTAGTCA
AGTTTAACTGGTGTCCACAGAGGACATGGTTTAACTGGAATTCGTCAAGCCTC
TGGTTCTAATTTCTCATTTGCAG 3'

In the C-BCD541 gene :

- for intron n° 6 :

AATTTTTAAATTTTTTGTAGAGACAGGGTCTCATTATGTTGCCCAGGGTGGTGTCAAGCTCCA
GGTCTCAAGTGATCCCCCTACCTCCGCCTCCCAAAGTTGTGGGATTGTAGGCATGAGCCACTG
CAAGAAAACCTTAACTGCAGCCTAATAATTGTTTTCTTTGGGATAACTTTTAAAGTACATTAA
AAGACTATCAACTTAATTTCTGATCATATTTTGTTGAATAAAATAAGTAAAATGTCTTGTGAA
CAAAATGCTTTTTAACATCCATATAAAGCTATCTATATATAGCTATCTATATCTATATAGCTA
TTTTTTTTAACTTCCTTTTATTTTCCTTACAG*

- for intron n° 7 :

*GTAAGTCTGCCAGCATTATGAAAGTGAATCTTACTTTT
GTAAAACTTTATGGTTTGTGGAAAACAAATGTTTTTGAACAGTTAAAAAGTTCAGATGTTAGA
AAGTTGAAAGGTTAATGTAAAACAATCAATATTAAAGAATTTTGATGCCAAAACTATTAGATA
AAAGGTTAATCTACATCCCTACTAGAATTCTCATACTTAACTGGTTGGTTGTGTGGAAGAAAC
ATACTTTCACAATAAAGAGCTTTAGGATATGATGCCATTTTATATCACTAGTAGGCAGACCAG
CAGACTTTTTTTTATTGTGATATGGGATAACCTAGGCATACTGCACTGTACACTCTGACATAT
GAAGTGCTCTAGTCAAGTTTAACTGGTGTCCACAGAGGACATGGTTTAACTGGAATTCGTCAA
GCCTCTGGTTCTAATTTCTCATTTGCAG*

In a preferred embodiment of the invention, the gene of the invention is capable of hybridizing in stringent conditions with the sequence of Figure 3 used as probe.

As hereabove written, the invention further relates to a variant of the SMN gene, which variant is a C-BCD541 gene having a cDNA sequence corresponding to the sequence of Figure 2.

The invention also relates to cDNA sequences such as obtained from one of the above genes. Such cDNA sequences are disclosed in Figures 2 and 3. Both of these cDNA sequence are capable of encoding a protein comprising the amino acid sequence described on Figure 1.

Despite this capacity to encode for such a protein, the inventors have noted that the C-BCD541 gene is able to produce in vivo this protein or is not able to produce it in a sufficient quantity due to the abnormal splicing of the gene during the transcription. Thus, the presence of the C-BCD541 gene does not enable to correct in vivo the deficiency (deletion, mutation,...) of the T-BCD541 gene responsible for the motor neuron diseases of the SMA type or other motor neuron disorders.

In a particular embodiment, the invention relates also to a nucleotide sequence comprising nucleotides 34 to 915 of the sequence of Figure 3, or to a sequence comprising nucleotides 34 to 915 of the sequence of Figure 2.

These nucleotide sequences correspond to the coding sequence of respectively the T-BCD541 gene and C-BCD541 gene.

The introns of the hereabove described genes are also included in the application. Especially introns 6 and 7 have respectively the following sequences :

For the T-BCD541 gene :

- Intron 6 :

5' AATTTTTAAATTTTTTGTAGAGACAGGGTCTCATTATGTTGCCCAGGGTG
GTGTCAAGCTCCAGGTCTCAAGTGATCCCCCTACCTCCGCCTCCCAAAGTTGT
GGGATTGTAGGCATGAGCCACTGCAAGAAAACCTTAACTGCAGCCTAATAATT
GTTTTCTTTGGGATAACTTTTAAAGTACATTAAAAGACTATCAACTTAATTTC
TGATCATATTTTGTTGAATAAAATAAGTAAAATGTCTTGTGAACAAAATGCTT
TTTAACATCCATATAAAGCTATCTATATATAGCTATCTATGTCTATATAGCTA
TTTTTTTTAACTTCCTTTTATTTTCCTTACAG 3'

- Intron 7 :

5' GTAAGTCTGCCAGCATTATGAAAGTGAATCTTACTTTTGTAAAACTTTAT
GGTTTGTGGAAAACAAATGTTTTTGAACAGTTAAAAAGTTCAGATGTTAAAAA
GTTGAAAGGTTAATGTAAAACAATCAATATTAAAGAATTTTGATGCCAAAACT
ATTAGATAAAAGGTTAATCTACATCCCTACTAGAATTCTCATACTTAACTGGT
TGGTTATGTGGAAGAAACATACTTTCACAATAAAGAGCTTTAGGATATGATGC
CATTTTATATCACTAGTAGGCAGACCAGCAGACTTTTTTTTATTGTGATATGG
GATAACCTAGGCATACTGCACTGTACACTCTGACATATGAAGTGCTCTAGTCA
AGTTTAACTGGTGTCCACAGAGGACATGGTTTAACTGGAATTCGTCAAGCCTC
TGGTTCTAATTTCTCATTTGCAG 3'

For the C-BCD541 gene :

- Intron 6 :

AATTTTTAAATTTTTTGTAGAGACAGGGTCTCATTATGTTGCCCAGGGTGGTGTCAAGCTCCA
GGTCTCAAGTGATCCCCCTACCTCCGCCTCCCAAAGTTGTGGGATTGTAGGCATGAGCCACTG
CAAGAAAACCTTAACTGCAGCCTAATAATTGTTTTCTTTGGGATAACTTTTAAAGTACATTAA
AAGACTATCAACTTAATTTCTGATCATATTTTGTTGAATAAAATAAGTAAAATGTCTTGTGAA
CAAAATGCTTTTTAACATCCATATAAAGCTATCTATATATAGCTATCTATATCTATATAGCTA
TTTTTTTTAACTTCCTTTTATTTTCCTTACAG*

- Intron 7 :

```
                          GTAAGTCTGCCAGCATTATGAAAGTGAATCTTACTTTT
GTAAAACTTTATGGTTTGTGGAAAACAAATGTTTTTGAACAGTTAAAAAGTTCAGATGTTAGA
AAGTTGAAAGGTTAATGTAAAACAATCAATATTAAAGAATTTTGATGCCAAAACTATTAGATA
AAAGGTTAATCTACATCCCTACTAGAATTCTCATACTTAACTGGTTGGTTGTGTGGAAGAAAC
ATACTTTCACAATAAAGAGCTTTAGGATATGATGCCATTTTATATCACTAGTAGGCAGACCAG
CAGACTTTTTTTTTATTGTGATATGGATAACCTAGGCATACTGCACTGTACACTCTGACATAT
GAAGTGCTCTAGTCAAGTTTAACTGGTGTCCACAGAGGACATGGTTTAACTGGAATTCGTCAA
GCCTCTGGTTCTAATTTCTCATTTGCAG*
```

The invention further encompasses a nucleotide sequence, characterized in that it comprises at least around 9 nucleotides and in that it is comprised within a sequence which has been described above or in that it hybridizes with a sequence as described above in hybridization conditions which are determined after choosing the oligonucleotide.

For the determination of the hybridization conditions, reference is made to the hybridization techniques for oligonucleotides probes such as disclosed in Sambrook et al, Molecular Cloning, a Laboratory Manual, 2nd edition, 1989.

The sequences of the invention are either DNA (especially genomic DNA or cDNA or synthetic DNA) or RNA. They can be used as probes for the detection of the T-BCD541 or C-BCD541 genes or as primers for the amplification of genomic DNA present in a biological sample.

Preferred primers are those comprising or relating to the following sequences :

a) 5' AGACTATCAACTTAATTTCTGATCA 3' (R 111)

b) 5' TAAGGAATGTGAGCACCTTCCTTC 3' (541C770)

The above primers are characteristic of exon 7 of the T-BCD541 gene.

(c) GTAATAACCAAATGCAATGTGAA (541C960)

(d) CTACAACACCCTTCTCACAG (541C1120)

The above primers are characteristic of exon 8 of the T-BCD541 gene.

The primers used by pairs can form sets for the amplification of genomic DNA in order to detect motor neuron diseases.

Inverted complementary sequences with respect to the above primers can also be used.

Preferred sets of primers are the following :

- a pair of primers contained in the sequence comprising nucleotides 921 to 1469 of the sequence of Figure 3 and/or
- a pair of primers comprising the following sequences :

5' AGACTATCAACTTAATTTCTGATCA 3'

5' TAAGGAATGTGAGCACCTTCCTTC 3'

Another preferred set of primers comprises :
- a pair of primers having the following sequences :

5' AGACTATCAACTTAATTTCTGATCA 3'

5' TAAGGAATGTGAGCACCTTCCTTC 3'

- a pair of primers having the following sequences :

5' GTAATAACCAAATGCAATGTGAA 3' and/or

5' CTACAACACCCTTCTCACAG 3'

From a general point of view for the detection of divergence in exon 7, between the T-BCD541 and C-BCD541 genes oligonucleotide primers can be selected in the fragment 5' from the divergence and within exon 7 or intron 7.

Other primers that can be used for SSCP analysis for diagnostic purposes are selected from amongst the following :

5'EXON 1 **121md/121me** **Size:170 bp**
121MD 5' AGG GCG AGG CTC TGT CTC A
121ME 5' CGG GAG GAC CGC TTG TAG T

EXON1 **121ma/121mf** **Size:180 bp**
121MA 5' GCC GGA AGT CGT CAC TCT T
121MF 5' GGG TGC TGA GAG CGC TAA TA

EXON2A **ex2A5/Ex2A3** **Size:242 bp**
EX2A5 5' TGT GTG GAT TAA GAT GAC TC
EX2A3 5' CAC TTT ATC GTA TGT TAT C

EXON2B **Ex2B5/EX23** **Size:215 bp**
EX2B5 5' CTG TGC ACC ACC CTG TAA CAT G
EX23 5' AAG GAC TAA TGA GAC ATC C

EXON3 **SM8C/161CR2** **Size:238 bp**
SM8C 5' CGA GAT GAT AGT TTG CCC TC
161CR2 5' AG CTA CTT CAC AGA TTG GGG AAA G

SM8D/C260 **Size:150 bp**
SM8D 5' CTC ATC TAG TCT CTG CTT CC
541C260 5' TGG ATA TGG AAA TAG AGA GGG AGC

EXON4	**SM3CA/C460**	**Size:150 bp**
SM3CA		5' CAC CCT TAT AAC AAA AAC CTG C
541C460		5' GAG AAA GGA GTT CCA TGG AGC AG
	SM3CB/C380	**Size:180 bp**
SM3CB		5' GAG AGG TTA AAT GTC CCG AC
541C380		5' GTG AGA ACT CCA GGT CTC CTG G
EXON5	**EX55/C590**	**Size:254 bp**
EX55		5' TGA GTC TGT TTG ACT TCA GG
541C590		5' GAA GGA AAT GGA GGC AGC CAG C
	EX53/C550	**Size:168 bp**
EX53		5' TTT CTA CCC ATT AGA ATC TGG
541C550		5' CCC CAC TTA CTA TCA TGC TGG CTG
EXON6	**164C25/C849**	**Size:143 bp**
164C25		5' CCA GAC TTT ACT TTT TGT TTA CTG
541C849		5' ATA GCC ACT CAT GTA CCA TGA
	EX63/C618	**Size:248 bp**
EX63		5' AAG AGT AAT TTA AGC CTC AGA CAG
541C618		5' CTC CCA TAT GTC CAG ATT CTC TTG 3'
EXON7	**R111/C770**	**Size: 200 bp**
R111		5' AGA CTA TCA ACT TAA TTT CTG ATC A
541C770		5' TAA GGA ATG TGA GCA CCT TCC TTC
	R111/C261	**Size:244 bp**
R111		5' AGA CTA TCA ACT TAA TTT CTG ATC A
164C261		5' GTA AGA TTC ACT TTC ATA ATG CTG

INTRON7	**164C45/164C265 Size:220 bp**	
164C45		5' CTT TAT GGT TTG TGG AAA ACA 3'
164C265		5' GGC ATC ATA TCC TAA AGC TC
EXON8	**C960/C1120 Size: 186 bp**	
541C960		5'GTA ATA ACC AAA TGC AAT GTG AA
541C1120		5'CTA CAA CAC CCT TCT CAC AG
	164C140/C920	
164C140		5' GGT GTC CAC AGA GGA CAT GG
541C920		5' AAG AGT TAA CCC ATT CCA GCT TCC

The invention also concerns antisense DNA or RNA, capable of hybridizing with the C-BCD541 gene and particularly to the intron sequences, especially with the fragment of the introns which differ from the corresponding part in the T-BCD541 gene.

The invention also relates to a protein comprising the amino acid sequence of Figure 1, or to a protein having the amino acid sequence of Figure 8.

The protein relating to the sequence of Figure 1 can be used in a composition for the treatment of motor neuron diseases, via oral, intra-muscular, intravenous administration, or via administration in the spinal cord fluid.

The invention further provides a kit for the in vitro diagnosis of motor neuron diseases, comprising :

- a set of primers as described above ;
- reagents for an amplification reaction ; and
- a probe for the detection of the amplified product.

According to another embodiment of the invention, a kit for the detection of the motor neuron diseases containing a hybridization probe as described above is provided.

Oligonucleotide probes corresponding to the divergences between the genes can be used.

The diagnosis can be especially directed to SMA motor neuron pathology.

The invention also concerns cloning or expression vectors comprising a nucleotide sequence as defined above. Such vectors can be, for example, plasmids, cosmids, phages, YAC, pYAC, and the like. Preferably, such a vector has a motor neuron tropism. Especially for the purpose of defining means for gene therapy, it can be chosen among poliovirus vector, herpes virus, adenovirus, retrovirus vectors, synthetic vectors and the like.

Within the scope of the invention are contemplated further recombinant sequences. The invention also concerns recombinant host cells, i.e., yeasts, CHO cells, baculovirus, bone marrow cells, *E. coli*, fibroblasts-epithelial cells, transformed by the above recombinant sequences.

The invention also relates to a method for detecting motor neuron disorders including spinal muscular atrophy, amyo trophoc lateral sclerosis and primary lateral sclerosis, said method comprising the steps of :

(a) extracting DNA from a patient sample ;

(b) amplifying said DNA with primers as described above ;

(c) subjecting said amplified DNA to SCCP ;

(d) autoradiographing the gels ; and

(e) detecting the presence or absence of the motor neuron disorder.

Steps (c) and (d) can be replaced by a step of digestion with Bsrl enzyme or with any other enzyme capable of recognizing specifically the divergence of the genes or mismatches in genes, or by sequencing.

The invention also relates to a method for detecting spinal muscular atrophy, said method comprising the steps of :

(a) extracting DNA from a patient sample ;

(b) hybridizing said DNA with a DNA probe comprising all or part of the cDNA sequence of Figure 3 or of Figure 2 under stringent conditions; and

(c) detecting the hybrids possibly formed.

The invention also relates to a method for detecting arthrogryposis multiplex congenita, said method comprising the steps of :

(a) extracting DNA from a patient sample ;

(b) amplifying said DNA via PCR using unlabeled primers from exon 7 and exon 8 of the SMN gene ;

(c) subjecting said amplified DNA to SCCP ;

(d) autoradiographing the gels ; and

(e) detecting the presence or absence of arthrogryposis multiplex congenita.

Yet another method to detect arthrogryposis multiplex congenita concerns dinucleotide Repeat Polymorphism Analysis using genotyping markers C272 and C212 after PCR amplification.

The present invention further concerns polyclonal antiserum or monoclonal antibodies directed to the protein of Figure 1, the protein of Figure 8 or the protein of Figure 12.

Yet another aspect of the present invention is directed to the use of the entire or partial nucleotide sequence of SMN as a probe to detect SMA as well

as to indetify and clone genes related to SMN gene motor neuron in animals or organisms.

Yet another aspect of the present invention is the use of the SMA protein to produce polyclonal and monoclonal antibodies, which antibodies may be used to detect and diagnose SMA.

In another aspect, polyclonal rabbit antiserum were generated against synthetic peptides corresponding to the amino acid sequence of Figures 1, 8 and 12, including the amino acid terminus and the carboxy terminus.

Accordingly, in one of its process aspects, the present invention relates to the detection of SMA in patients having SMA or related motor neuron disorders such as AMC, ALS and PLS.

Yet another aspect of the present invention is to administer the SMN gene part thereof, cDNA or oligonucleotides to patients who are either lacking the gene or have a genetically defective gene as such or after incorporation into engineered viruses or vectors.

These and other aspects of the present invention will be discussed in detail below in the preferred embodiments of the present invention.

BRIEF DESCRIPTION OF THE DRAWINGS

Fig. 1 is the amino acid sequence of the SMN coding region of the clone T-BCD541.

Fig. 2 is th nucleotide sequence of the SMN coding region as well as the 5' and 3' flanking regions of clone C-BCD541 ; the coding region is underlined.

Fig. 2B contains the sequence starting from intron 6 up to exon 8 of the C-BCD541 gene. The underlined sequences are those of exons 7 and 8. Sequences of introns 6 and 7 can be chosen as oligonucleotides to amplify the cDNA region allowing the distinction, within exon 7, between the T-BCD541 gene and the C-BCD541 gene. The position of the divergent nucleotides between the T-BCD541 and C-BCD541 cDNA are in italics.

Fig. 3A is the nucleotide sequence of the SMN coding region as well as the 5' and 3' flanking regions of clone T-BCD541. The coding sequences are underlined. The numbers of the exons are indicated on the sequence. Asteriks indicate the beginning of each exon. The nucleotides which are indicated in italics are those which differ between the C-BCD541 and the T-BCD541 genes.

Fig. 3B represents the sequence from intron 6 up to the end of exon 8 of the T-BCD541 gene. The sequence of exons 7 and 8 is underlined.

Fig. 4 is the nucleotide sequences of the markers C212, C272, C171, AFM157xd10, and C161.

Fig. 5 represents various probes utilized in the present invention revealing several loci that the probes hybridized to.

Fig. 6 represents the telomeric element containing the survival SMN gene.

Fig. 7 represents the marked decrease of gene dosage with probe 132SEII, mapping close to this.

Fig. 8 represents the amino acid sequence of the truncated SMN protein.

Fig. 9 is a schematic representation of the genomic structure of the human SMN gene. The designations and positions of genomic clones are shown above the figure. L-132, L-5, and L-13 depict the genomic clones spanning the entire

SMN gene, while L-51 spans part of exon 1. Micro satellites and DNA markers are indicated above the genomic map. B, H, and E mean BglII, HindIII and EcoRI, respectively. C212, p322, C272, 132SEII and C171 represent various markers. 1, 2a, 2b, 3, 4, 5, 6, 7, and 8 represent exons of the SMN and C-BCD541 genes. The entire sequence of L-132 is obtained by PCR amplification from exon 1 to exon 2A.

Fig. 10 represents the nucleotide sequence and amino acid sequence of the entire human SMN gene including the introns and exons. Translated nucleotide sequences are in upper case, with the corresponding amino acids shown below that. The polyadenylation signal is in bold face. Arrowheads indicate the position of the single base differences between SMN and C-BCD541 genes in Introns 6 and 7 and exons 7 and 8. Italic letters indicate the position of the oligonulcoeitdes chosen for the detection of divergences in intron 7. (*) indicates the position of the stop codon.

Fig. 11 represents the nucleotide sequence upstream of the coding region of the human SMN gene and illustrates the presence of putative binding sites for the transcription factors of AP-2, GH-CSE2, DTF-1, E4FI, HINF-A, H4TF-1, β-IFN and Spl. Bold letters indicate the dinucleotide repeat (CA) corresponding to the C272 markers.

Fig. 12 represents the nucleotide and amino acid sequences of Mouse SMN cDNA. (*) indicates the position of the stop codon.

Fig. 13 represents a comparative analysis of the amino acid sequence of human SMN (above) and mouse SMN (below).

Fig. 14 illustrates the genetic analysis of family 6. Lane A shows evidence of inherited maternal deletion seen with the microsatellite marker C272 as the proband inherited only allele from the father. Lanes B and C represent SSCP analysis of PCR-amplified exons 7 (lane B) and 8 (lane C) of SMN (closed

arrowheads) and its centromeric copy (open arrowheads). "F" represents the father, "M" the mother, and "A" the affected infant.

Fig. 15 illustrates the band shifts on single strand confirmation polymorphism (SSCP) analysis of the PCR amplified intron 7 and permitted indetification of SMN (closed arrowheads) and its centromeric counterpart C-BCD541 (open arrowheads).

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS OF THE INVENTION

As used herein, the term "contig" means overlapping nucleotide sequences.

Previous studies by means of linkagec analysis have shown that all three forms of spinal muscular atrophy map to chromosome 5q11.2-q13.3. (L.M. Brzustowicz et al, Nature, **344**, 540 (1990) ; J. Melki et al, Nature, **345**, 823 (1990) ; J. Melki et al, Lancet, **336**, 271 (1990). A yeast artificial chromosome (YAC) contig of the 5q13 region spanning the disease locus was constructed that showed the presence of low copy-repeats in this region. Allele segregation was analyzed at the closest genetic loci detected by markers derived from the YAC contig (C212, C272 and C161) in 201 SMA families. These markers revealed two loci (C212, C272) or three loci on the 5q13 region (C161). Inherited and de novo deletions were observed in 9 unrelated SMA patients. Moreover, deletions were strongly suggested in at least 18% of SMA type I patients by the observation of marked heterozygosity deficiency for the loci studied. These results indicated that deletion events are statistically associated with the severe form of SMA.

By studying all polymorphic DNA markers derived from the YAC contig, it was observed that the smallest rearrangement occured within a region bordered by loci detected by C161 and C212-C272 and entirely contained in a 1.2-Mb

YAC clone 903D1. See, for example, French Patent Application No. 9406856 incorporated herein by reference.

The present invention characterized the small nested critical SMA region of about 140 Kb by a combination of genetic and physical mapping in SMA patients. This region suggested a precise location for the SMA gene and therefore, a limited region within which to search for candidate genes. The present invention identified a duplicated gene from the 5q13 region. One of them (the telomeric gene) is localized within the critical region. Moreover, this gene was lacking in 213 out of 230 (92.2%) or interrupted in 13 out of 230 (5.6%) SMA patients. In patients where the telomeric gene is not lacking or interrupted, deleterious mutations indicated that this telomeric gene, termed survival motor-neuron (SMN) gene, is the chromosome 5 SMA-determining gene.

The SMN gene was discovered using a complex system of restriction mapping, distinguishing the E^{Tel} from the E^{Cen} by Southern blot, and the determination of the differences between the E^{Tel} in SMA patients by genetic and physical mapping. After confirming the location of the SMN gene, a phage contig spanning the critical region of the telomeric element was constructed to identify specific clones containing the SMN gene.

Analysis of the SMN gene in SMA patients compared with those of normal patients revealed either the SMN gene was either lacking or truncated in 98% of SMA patients or had combined mutations not present in normal control patients.

To identify a large inverted duplication and a complex genomic organisation of the 5q13 region, long-range restriction mapping using pulsed field gel electrophoresis (PFGE) of the YAC contig was performed.

YACs were ordered by comparing their haplotypes with that of the human donor at the polymorphic loci detected markers C212, C272, C171 and C161 (Fig. 4).

The restriction enzymes SacII, BssHII, SfiI, EagI and XhoI were used to digest the YACs containing the telomeric loci detected by markers C212, C272, C171 and C161 (YAC clone 595C11), the centromeric loci detected by these markers (YAC clones 121B8, 759A3, 278G7) or both (YAC clones 903D1 and 920C9). Lambda phage libraries of YACs 595C11, 121B8 and 903D1 were constructed and subclones from phages containing markers C212 (p322), C272 (132SE11), C161(He3), AFM157xd10(131xb4) and CMS1 (p11M1) were used as probes for PFGE analysis. Fig. 5 shows that probes 132SE11, 11P1 and p322 revealed two loci, and probe He3 revealed 4 loci on the YAC contig, whereas probe 131xb4 revealed several loci on 5p and 5q13. The restriction map (Figure 6) showed that the 5q13 region contained a large inverted duplication of an element (E) of at least 500 Kb, termed E^{Tel} and E^{Cen} for the telomeric and centromeric elements, respectively.

The PFGE analysis of SMA and control individuals revealed a high degree of variability of restriction fragments which hampered the distinghishment of E^{Tel} from the E^{Cen} and the recognition of abnormal restriction fragments in SMA patients.

In order to distinguish between the E^{Tel} and the E^{Cen}, a Southern blot analysis was then performed. The Southern blot was performed by the methods described in Sambrook et al, *supra*.

More specifically, DNA from YAC clones, controls and SMA patients was digested with restriction enzymes SacI, KpnI, MspI, PstI, PvuII, EcoRI, HindIII, BglII and XbaI for Southern blotting and hybridized with clones 132SE11, 11p1, He3, 131xb4 and p322 as probes. None of the probes except one (He3) detected a difference between the two duplicated elements. Three HindIII restriction fragments of 12, 11 and 3.7 Kb were detected by probe He3. A 12 Kb HindIII restriction fragment was detected in YAC clones 754H5 and 759A3, indicating that this fragment corresponded to the most centromeric locus in the E^{Cen}.

Conversely, a 11 Kb HindIII fragment was detected in YACs clones 595C11, 903D1 and 920C9 indicating that this fragment corresponded to a single locus on the E^{Tel}. Finally, a 3.7 Kb HindIII fragment was noted in non-overlapping YACs containing either E^{Tel} or E^{Con}, indicating that this fragment corresponded to two different loci. Similar results were obtained with SacI and KpnI. The three restriction fragments detected by He3 were observed on the monochromosomal hybrid HHW105 (Carlock, L.R. et al, Am. J. of Human Genet., 1985, Vol. 37, p. 839) and in 30 unrelated, healthy individuals, confirming that these fragments were not due to polymorphisms. The Southern analysis results allowed one to distinguish E^{Tel} from the E^{Cen} in both controls and SMA patients.

Thus, once the E^{Tel} from the E^{Cen} was distinguished, it was necessary to determine the differences between the E^{Tel} in SMA patients and those of the normal control. This was done by using genetic and physical mapping. This genetic and physical mapping identified genomic rearrangements in the telomeric element of E^{Tel} of SMA patients.

It was previously shown that 9 out of 201 (9/201) SMA patients displayed large-scale deletions encompassing either one or the two loci detected by markers C212 and C272 on one mutant chromosome (J. Melki et al, Science, **264**, 1474 (1994)). On the other hand, 22 out of 30 (22/30) patients born to consanguineous parents including 13 out of 14 (13/14) type I and 9 out of 10 (9/10) type III SMA, were homozygous by descent for the most closely flanking polymorphic markers.

The genomic DNA of the 9 patients harboring large scale deletions and the 22 consanguineous patients displaying homozygosity by descent were digested with HindIII for Southern blotting and hybridized with probe He3. The 11 Kb fragment revealed by probe He3 was absent in 12 out of 13 (12/13) consanguineous type I patients. In 2 out of 12 (2/12), the deletion also involved the 3.7 Kb fragment. By contrast, the 11 Kb fragment was absent in 1 out of 8 (1/8) consanguineous type III patients only. Consistently, the 11 Kb HindIII

fragment was absent in 4 out of 9 (4/9) patients harboring large scale deletions on one mutant chromosome. Of particular interest was the absence of the 11 Kb fragment in the patient harboring a deletion of one of the two loci detected by markers C212 and C272.

When analyzed together, these observations provided evidence for genomic rearrangements of E^{Tel} in SMA patients and supported the location of the SMA gene centromeric to the locus revealed by the 11 Kb HindIII fragment, since all consanguineous type III patients but one were not deleted for this locus.

In order to characterize the centromeric boundary of the genomic rearrangement in the disease, the allele segregation at loci detected by marker C272 in consanguineous SMA patients was analyzed. All consanguineous SMA type I patients had one single PCR amplification product, compared with 0 out of 60 controls. This marked heterozygosity deficiency was due to deletion of one of the two loci detected by C272, as indicated by the marked decrease of gene dosage with probe 132SE11, mapping close to this marker. By contrast, 7 out of 9 (7/9) consanguineous type III SMA patients had two C272 amplification products inherited from both parents, indicating homozygosity at each locus detected by marker C272. Moreover, no gene dosage effect was observed with probe 132SE11 indicating the absence of deletion involving the locus detected by C272 in type III consanguineous patients.

Assuming that the same locus is involved in all three types of SMA, these results indicate that the disease causing gene is distal to the telomeric locus detected by C272.

These studies place the SMA gene within the telomeric element E^{Tel}, between the telomeric loci detected by markers C272 and He3 (11 kb HindIII fragment). Based on long-range restriction mapping using PGFE of the YAC contig, this critical region is entirely contained in a 140 Kb SacII fragment of YAC clone 903D1 (or 150 Kb SacII fragment of YAC clone 920D9).

After confirming that the SMN gene was located on a 140 Kb SacII fragment a phage contig spanning the critical region of the telomeric element was constructed in order to identify and characterize the SMN gene.

Phage clones containing markers C212, C272, C171 and C161 were isolated from the λ phage libraries constructed from YAC clones 595C11 and 903D1 and used as a starting point for bidirectional walking. A phage contig (60 Kb) surrounding markers C212, C272 and C171 was constructed based on the restriction map of the phage clones (Fig. 6).

To identify genes in the contig, the following three stategies were used :

1) a search for interspecies-conserved sequences was conducted ;
2) exon trapping method was performed ; and
3) direct cDNA selection was performed. The genomic probe 132SE11, derived from the phage containing the marker C272, gave positive hybridization signals with hamster DNA indicating the presence of interspecies-conserved sequences. The screening of a λgt10 human fetal brain cDNA library with probe 132SE11 resulted in the selection of 7 overlapping λ clones spanning 1.6 kbp. Sequence analysis of the clones revealed a 882 bp open-reading frame (ORF) and a 580 bp non-coding region. A 1.5 kbp clone (BCD541) contained the entire coding sequence and most of the 3' non-coding region. The 3' end of the cDNA along with its poly(A)$^+$ tail was obtained by PCR-amplification of a lymphoblastoid cell line cDNA library.

Two cDNA clones lacked nucleotides 661 to 755, suggesting that an alternative splicing might have occured. Northern blot analysis of poly(A)$^+$ RNA from various tissues including heart, brain, liver, muscle, lung, kidney and pancreas, revealed the presence of a widely expressed 1.7 kb transcript. The ORF encodes a putative protein of 294 amino acids with a predicted molecular weight of approximately 32 Kd.

A homology search using the FASTA and BLAST networks failed to detect any homology at either the nucleotide or the amino acid level.

To further distinguish whether there was any duplication of the BCD541 gene in the 5q13 region, BCD541 cDNA was used as a probe for Southern blot and PFGE analysis of YAC clones spanning the disease locus.

Specific hybridization with non-overlapping YACs containing either the E^{Cen} only (YAC clones 759A3, 121B8 and 278G7), or containing the E^{Tel} only (YAC clone 595C11) provided evidence for duplication of the BCD541 gene. Each gene encompassed approximately 20 kb and displayed an identical restriction pattern. Evidence for head to head orientation of the two genes was derived from the location of the SacII and EagI restriction sites of the non-overlapping YAC clones containing either E^{Cen} or E^{Tel}, following hybridization experiments with probes BCD541 and p322 which flank the SacII and EagI sites of each element.

In order to look for divergences in the two copies of the BCD541 gene, the organization of the telomeric gene was characterized and compared to that of the centromeric counterpart. Genomic sequence analysis revealed that the telomeric BCD541 gene is composed of 8 exons (Fig. 3). However, it is now known that the previously known exon 2 is composed of 2 exons separated by an additional intron as set forth in Fig. 10, therefore the SMN gene is composed of 9 exons.

Starting from either the centromeric or telomeric gene loci (in YAC clones 121B8 and 595C11, respectively), PCR-amplification and sequence of each exon and their flanking regions revealed five discrepancies between the centromeric and the telomeric BCD541 genes. The first one is a conservative substitution in exon 7 (codon 280) specific for the telomeric (TTC) or the centromeric BCD541 gene (TTT). The second one, located in the 3' non-coding region (exon 8 nucleotide n° 1155) is specific for the telomeric (TGG) or the centromeric

BCD541 gene (TGA). Three other single base substitutions were observed in the sixth and seventh introns.

The observation of both versions of each exon (exon 7 and 8) on either YAC clones containing both gene loci (YAC clone 920C9) or the monochromosomal hybrid HHW105 demonstrated that these substitutions are neither allelic nor due to polymorphisms. Band shifts on SSCP analysis of amplified exons 7 and 8 allowed an easy distinction of the telomeric (T-BCD541) and centromeric genes (C-BCD541) in both controls and SMA patients. All the unrelated healthy controls tested (n=75) harbored the T-BCD541 gene as determined by SSCP analysis of exons 7 and 8 (100%). Most of them (89.3%) also harbored the C-BCD541 gene but 8 out of 75 (8/75) (10.7%) lacked the C-BCD541.

A total of 230 SMA patients were tested for single base substitutions detected in exons 7 and 8 by SSCP method after PCR-amplification of genomic DNA. Among them, 103 belonged to type I, 91 to type II, and 36 to type III. Interestingly, 213 out of 230 SMA patients (92.6%) lacked the T-BCD541 gene on both mutant chromosomes compared with 0 out of 75 controls (0%). Moreover, 13 out of 230 SMA patients (5.6%) lacked the T-BCD541 gene for exon 7 on both mutant chromosomes but retained the T-BCD541 gene for exon 8 compared with 0 out of 75 controls (0%). Finally, only 4 out of 230 SMA patients (1.7%) harbored the T-BCD541 gene as determined by SSCP analysis of exons 7 and 8.

These results show that the T-BCD541 gene is either lacking or truncated in 98% of SMA patients. In addition, these data support the view that the disease gene is located between the telomeric locus detected by C272 and exon 8 of the T-BCD541 gene. Therefore, according to the overlapping restriction map of the phage contig, the critical region is entirely contained in 20 kb, suggesting that the telomeric BCD541 gene is the chromosome 5 SMA-determining gene.

In order to demonstrate that the T-BCD541 gene is responsible for SMA, point mutations in the 4 SMA patients in whom no rearrangement of the T-BCD541 gene had been observed were searched. Direct sequencing of PCR amplification products of each exon with their flanking regions was performed in the four patients.

A 7 bp deletion in the 3' splice acceptor site of intron 6 (polypyrimidine tract) was found in patient SA. Sequence analysis of exon 7 flanking the deleted intron, recognized the sequence specific for the T-BCD541 gene. Moreover, the non-deleted PCR-product corresponding to the same region, harbored the sequence specific for the C-BCD541 suggesting that the other mutant allele lacked the T-BCD541 gene.

In patient BI, a 4 bp deletion in the 5' consensus splice donor site of intron 7 was found. This deletion occured on the T-BCD541 gene as determined by sequence analysis of the flanking exon 7.

In patient HU, a point mutation in codon 272 (TAT→TGT) was found. This mutation changed a Tyrosine to Cysteine. The patient was heterozygous for the mutation, presumably carrying a different SMA mutation on the other allele. All three mutations observed in patients SA, HU and BI were not detected in 100 normal chromosomes ruling out rare polymorphisms.

A different splicing of exon 7 distinguished the C-BCD541 from the T-BCD541 gene using reverse transcription-based PCR. Eleven SMA patients were selected for the analysis of their transcripts by Northern blot or reverse transcription-based PCR amplification. Eight of them belonged to type I, 1 to type II and 2 to type III. SSCP analysis of genomic DNA showed an absence of T-BCD541 gene in 10 patients and one patient (SA) had C-BCD541 and T-BCD541 genes for both exons 7 and 8. Six unrelated controls who harbored both C-BCD541 and T-BCD541 genes and 2 controls with only T-BCD541 gene were included in the present study.

The expression of this gene in lymphoblasts made it possible to analyze the BCD541 transcripts in cell lines derived from controls and SMA patients. Northern blot analysis of RNA from lymphoblastoid cell lines showed the presence of a 1.7 kb mRNA in all samples. None of the SMA patients showed a transcript of altered size. It was observed that a reduced level of transcripts was obtained when compared to the expression of the β-actine gene in 3 out of 4 type I SMA patients. Normal mRNA level were found for the other SMA probands.

Since the Northern blot analysis revealed the presence of a transcript in SMA patients who had the C-BCD541 gene only for both exons 7 and 8 as determined by SSCP analysis, these results indicated that both C-BCD541 and T-BCD541 genes were expressed. To prove whether both BCD541 genes were expressed, RT-based PCR amplification of RNA isolated from the lymphoblastoid cell lines from controls and SMA patients was used. Direct sequencing of PCR products flanking exons 7 and 8 revealed that patients who had C-BCD541 only displayed the sequence specific for the C-BCD541 gene. Controls who had both T-BCD541 and C-BCD541 genes, had two types of transcripts corresponding to both BCD541 genes. These results confirmed that both genes were expressed. In addition, 2 alternative splicings involving exon 5 or exon 7 that resulted in different transcripts were observed. The alternative splicing of exon 5 confirmed previous sequence data on the cDNA clones.

The analysis of the RT-PCR amplification products encompassing exons 6 to 8 showed that the spliced transcript keeping exon 7, was present in controls who had both C-BCD541 and T-BCD541 genes or controls who had the T-BCD541 gene only. Conversely, the alternative spliced transcript lacking exon 7 was observed in controls who had both genes, but not in controls who had the T-BCD541 gene only. These results indicated that the alternative spliced transcript lacking exon 7 was derived from the C-BCD541 gene only.

The transcript analysis of patient SA harboring a 7 bp deletion of the 3' splice acceptor site of intron 6 of the T-BCD541 gene revealed the presence of both spliced transcript keeping exon 7 and alternate spliced transcript lacking exon 7. Moreover, the sequence analysis of amplification products from the spliced transcript keeping exon 7, showed a sequence specific for the C-BCD541 gene (Fig. 2). These results demonstrated that the 7 bp deletion of intron 6 observed in patient SA was deleterious for the correct splicing of exon 7 of T-BCD541 gene only. In addition, because a differential splicing of exon 7 allowed one to distinguish the 2 BCD541 genes, this difference was analyzed among controls and SMA patients including patient SA. In controls, the amount of alternated spliced transcript lacking exon 7 was less abundant than that of spliced product keeping exon 7. Conversely, in SMA patients, the amount of alternated spliced transcript lacking exon 7 was equal or more abundant than that of spliced product keeping exon 7.

These results provide evidence for a difference between controls and SMA patients at the transcription level of these genes. The alternative spliced transcript lacking exon 7 resulted in a shorter ORF with a different C-terminus protein that might have effects on the protein function.

To further characterize the entire structure and organization of the human SMN gene, three genomic clones were isolated from a FIX II phage library derived from YAC clone 595C11 and screened with the full-length BCD541 cDNA (Fig. 2A) as a probe. After selecting several clones that hybridized to the probe, restriction mapping and Southern blot analysis indicated that phages L-132, L-5 and L-13 spanned the entire SMN gene.

These three phage clones were further subjected to sequencing using the Maxam-Gilbert or Sanger et al methods of sequencing disclosed in Sambrook et al *supra*.

The nucleotide and amino acid sequence of the entire SMN gene including exons and introns is set forth in Figure 10. The human gene is approximately 20 kb in length and consists of nine (9) exons interrupted by 8 introns as shown in Figure 10. The human SMN gene has a molecular weight of approximately 32 kDA.

Although it was thought that only one exon 2 was present in the SMN gene (see, Lefebvre et al, Cell, 80:155-165 (1995)), the sequencing data proved otherwise and the previously mentioned exon 2 in Lefebvre et al *supra* is in fact composed of 2 exons separated by an additional intron, as illustrated in Figures 9 and 10. To avoid confusion in the renumbering of exons, the 2 exons in exon 2 are now referred to as exon 2a and exon 2b.

All exon-intron bounderies displayed the consensus sequence found in other human genes and a polyadenylation consensus site is localized 550 bp downstream from the stop codon (Fig. 10).

Starting from either YAC clones 121B8 or 595C11 (which contain the C-BCD541 and SMN genes respectively, (see, Lefebvre et al, *supra*) PCR amplification and sequence analysis of the introns showed three differences between SMN and C-BCD541 in addition to those previously described (by Lefebvre et al, *supra*). These included a base change in intron 6 (-45bp/exon 7, atgt, telomeric; atat, centromeric) and two changes in intron 7 (+100bp/exon 7, ttaa, telomeric; ttag, centromeric and at position +214bp/exon 7, ttat, telomeric; ttgt, centromeric, Figure 10). The presence of both versions in a YAC clone containing both genes (YAC 920C9), and in the control population demonstrated that these substitutions are locus- specific rather than due to polymorphism. Band shifts on single strand conformation polymorphism (SSCP) analysis of the

PCR amplified intron 7 allowed SMN and its centromeric counterpart (C-BCD541) to be readily distinguished (see, Figure 15).

In order to identify sequences potentially important for promoter function, the organization of the region surrounding exon 1 of the SMN and C-BCD541 genes was characterized. Based on restriction mapping, Southern blot hybridization and PCR amplification, exon 1 and the C272 marker (D5F150S1, D5F150S2) were located in the same BglII-EcoRI restriction fragment of L-132 phage (Figure 9). PCR amplification using the C272f primer and a reverse primer chosen in exon 1 was performed and the amplified product was directly sequenced. Sequence analysis showed that the (CA) repeat corresponding to the C272 marker are located 463bp upstream from the putative ATG translation start site (Figure 11). Comparative sequence analyses showed no discrepancy between the 5' ends of the SMN gene and its centromeric counterpart (C-BCD541). In addition, sequence analysis showed the presence of putative binding sites for the following transcription factors: AP-2, GH-CSE2, DTF-1, E4F1, HiNF-A, H4TF-1, β-IFN, Sp1 (Figure 11 ; Faisst et al, Nucleic Acids Res., 20:3-26 (1992)).

Besides isolating and characterizing the human SMN gene, the mouse homologue of the SMN gene was also cloned. Cross-species conservation of human SMN gene with rodents has been shown in Lefebvre et al, *supra* and served to isolate the mouse SMN gene. Screening of a mouse fetal cDNA library using human SMN cDNA as a probe allowed the isolation of 2 overlapping mouse cDNA clones. Sequence analysis of the clones revealed an 864 bp open-reading frame (ORF) (Fig. 12). The ORF encodes a putative protein of 288

amino acids (Fig. 12) with an homology of 83% with human SMN amino acid sequence (Fig 13).

Either the isolated human or the mouse SMN, the gene can be inserted into various plasmids such as pUC18, pBr322, pUC100, λgHI, λ18-23, λZAP, λORF8, and the like. The methods for inserting genes into different plasmid vectors are described by Sambrook et al *supra*. Various microorganisms can be used to transform the vector to produce the SMN gene. For example, host microorganisms include, but are not limited to, yeast, CHO cells, E. coli, Bacillus subtilis and the like.

Once recombinantly produced, the human SMN protein or the mouse SMN protein can be further purified from the host culture by methods known in the art.

Besides recombinantly producing the SMN protein, the present invention also relates to the production of polyclonal and monoclonal antibodies. These methods are known in the art as evidenced by Sambrook et al *supra*. The monoclonal antibody can be obtained by the procedure of Kohler and Milstein, Nature, 256:495 (1975) ; Eur. J. Immunol., 6:511 (1976) or Harlow and Lane Antibodies, a Laboratory Manual, Cold Spring Harbor Laboratory, Cold Spring Harbor, New York (1988), and can be used, for example, in diagnosing SMA, as well as other motor neuron disorders.

Polyclonal rabbit antisera can also be generated against synthetic peptides corresponding to any part of the SMN amino acid sequence including the amino terminus and carboxy terminus. More specifically, the following peptides were synthesized based on the amino acid sequence set forth in Figure 1 :

N-terminal G G V P E Q E D S V L F R R G T C-terminal

S R S F G N K S D N I K P K

F R Q N Q K E G R C S H S L N

The synthetic peptide may be coupled to a carrier protein such as Keyhole limpet hemocyanin (KLH) through an amino- or carboxy-artificial cysteine residue that may be synthetically added to the desired sequence. The cysteine residue is used as a linker to couple the synthetic peptide to the carrier protein. The procedure utilized to couple synthetic peptides to KLH is described by Green et al, Cell, 28:477 (1982).

Approximately, 50-100 μg, preferably 100 μg of synthetic antigen is dissolved in buffer and emulsified with an equal volume of Freund's complete adjuvant. About .025 ml to 0.5 ml of emulsified antigen-adjuvant can be injected intramuscularly or intradermaly into a rabbit. Four to six weeks later, the rabbit is boosted and 20-40 ml of blood is drawn 7-10 days after each booster injection. The serum is then tested for the presence of antigen using RIA, ELISA or immunoprecipitation. The positive antibody fractions may then be purified, for example by absorption to protein A following the method of Goudswaald et al, Scand. J. Immunol., 8:21 (1978).

More specifically, about 20 to 50 μg of antigen, prepared either by the recombinant techniques set forth above or synthetically made antigen is diluted in about 100 μl of buffer and emulsified with an equal amount of Freund's complete adjuvant. About 30-60, preferably 50 μl of the emulsified antigen-adjuvant is injected subcutaneously at four sites into mice. Four to six weeks later, the mice are boosted with an intraperitoneal injection of about 100 μl containing 5-10 μg of antigen solubilized in buffer. The mice are bled from the mediam tail vein 7-10 days after the boaster injection and the serum is tested for antibody using standard methods. Blood is then drawn every 3-4 days until the antibody titer drops.

Tissue, plasma, serum, cerebral spinal fluid and the like can be used to detect SMA disease using the above-described monoclonal or polyclonal

antibodies via Western blot (1 or 2 dimensional) or ELISA. These methods are known in the art as described by Sambrook et al, *supra*.

A method for detecting SMA as well as in ALS, ACM, and PLS patients who possibly have these motor neuron disorders, is also encompassed by the present invention. This method involves extracting from a patient suspected of having SMA, DNA from a sample. This sample may include sera, plasma, cerebral spinal fluid and the like. After extracting the DNA by known methods in the art, primers that are derived from exons 7 and 8 of the SMN gene are used to amplify the DNA.

After amplification with the primer, the amplified product is subjected to SSCP (Single Strand Conformation Polymorphism).

The gels are then subjected to autoradiography to determine if SMA is present in the sample.

More specifically, it has recently been discovered that in twelve cases of arthrogryposis multiplex congenita (AMC) associated with SMA, 6 out of 12 patients lacked the SMN gene.

A total of twelve unrelated patients including eight males and four females of various geographic origins was selected for the study. The patients were chosen based on the criteria that these patients had :

(1) congenital joint contractures of at least two regions of the body (see, Stern, JAMA, 81:1507-1510 (1923)) ;

(2) generalized muscle weakness with muscular atrophy and areflexia without extraocular involvement ;

(3) electromyographic studies showed denervation and diminished motor action potential amplitude ; and

(4) muscle biopsies consistent with denervation with no evidence of storage material or other structural abnormalities (see, Munsat, Neuromuscular Disorders, 1:81 (1991)).

The study consisted of Dinucleotide Repeat Polymorphism Analysis and SMN gene analysis (see, Examples) based on DNA extracted from peripheral blood leukocytes, lymlphoblastoid cell lines or muscle tissue in all twelve patients.

The data from this study is summarized in Table 1 below.

The diagnosis was made at birth with an uniform phenotype characterized by a severe hypotonia, absence of movements except extraocular mobility and contractures of at least two joints. The number of affected joints and the severity of the postural defects varied from infant to infant, as set forth in Table 1. Decreased fetal movements were noted in 7 out of 12 (7/12) patients. Neonatal respiratory distress was observed in 9 out of 12 (9/12) patients and facial involvement associated with micrognathia was noted in 4 out of 12 (4/12) patients. Most of the cases, 8 out of 12 (8/12), died within the first month of life. Four infants are still alive. No family history was noted except in family 12 in which both the child and her father were affected suggesting an autosomal dominant form of AMC.

Table 1 shows that the SMN gene was lacking on both mutant chromosomes in 6 out of 12 (6/12) patients (cases 1-6). Among them, 3 out of 6 (3/6) patients had a large inherited deletion involving both loci detected by

markers C212 and C272 on one parental allele, the other parental carrying only one locus instead of the expected two, as shown in Figure 14.

Analysis of SMN exons did not reveal intragenic mutations in the patients whose SMN gene showed no deletions (cases 7-12). Genetic analysis showed that the disease gene in a family (case 9) was not linked to chromosome 5q13 as both the affected and healthy siblings carried the same 5q13 haplotype. These data strongly suggest that the patients whose SMN gene showed no deletions were not linked to the 5q13 SMA locus (cases 7-12).

Hitherto, arthrogryposis was regarded as an exclusion criterion in SMA (see, Munsat, *supra*). But the observation of SMN gene deletion in 6 out of 12 (6/12) patients (50%) strongly indicates that arthrogryposis of neurogenic origin is related to SMA and that this subgroup and SMA are allelic disorders. Yet, AMC of neurogenic origin is a genetically heterogeneous condition since the disease gene was not linked to SMN locus in 6 out of 12 (6/12) patients. Exclusion of chromosome 5q has also been shown in one family with two AMC-SMA patients, as described by Lunt et al, J. Med. Genet., 29:273 (Abstract) (1992).

Thus, by dinucleotide Repeat Polymorphism Analysis and SMN gene analysis, clinical diagnosis of AMC can be confirmed by the absence or interruption of the SMN gene. The present invention now provides methods to detect AMC either in live patients or in utero.

Yet another embodiment of the present invention is the detection of SMA using specific oligonucleotide probes based on the nucleotide sequence set forth in Figures 3, 10, or for the mouse SMA Figure 12. If a patient totally is lacking in the SMN gene, no hybridization to the specific probe will occur. The hybridization

conditions may vary depending upon the type of sample utilized. It is preferable to conduct such hybridization analysis under stringent conditions which are known in the art and defined in Sambrook et al *supra*. The oligonucleotide probes may be labeled in any manner such as with enzymes, radioactivity and the like. It is preferable to use radiolabeled probes.

In another embodiment of the present invention, the human SMN gene can be utilized in conjunction with a viral or non-viral vector for administration in vivo directly to the patients suffering from SMA or related motor neuron diseases or by administration in vitro in bone marrow cells, epithelial cells fibroplasts, followed by administration to the patient. See, for example Resenfeld et al, Science (1991) 252 , pp. 431 to 434.

The present invention provides a method of detecting SMN gene defects or the total lack of the SMN gene in a fetus. Amniotic fluid taken from the pregnant woman is subjected to SSCP analysis according to the methods of the present invention.

In order to further illustrate the present invention and advantages thereof, the following specific examples are given, it being understood that the same are intended only as illustration and in nowise limitative.

EXAMPLES

EXAMPLE 1

Construction of phage libraries from the 121B8, 595C11, and 903D1 YAC clone.

Total yeast DNA from YAC clone 595C11 contaiinng the telomeric loci detected by C212, C272 and C161, or YAC clone 121B8 containing the centromeric loci detected by the same markers or 903D1 YAC clone containing both loci was purified and partially digested with Sau3A. DNA in the size range of 12 to 23 kb was excised after 0.5% Seaplaque GTG agarose gel electrophoresis and precipitated with ethanol after β-agarase digestion. After partial fill-in of the Sau3A site, DNA was subcloned at the partially filled XhoI site of bacteriophage FIXIII (Stratagene). Clones of λ containing the microsatellite DNA markers C212 (L-51), C272 (L-51, L-132), C171 (L-5, L-13), C161 (595B1), 11M1 (L-11), AFM157xd10 (L-131) were digested either with EcoRI or HindIII or both and subcloned into pUC18 plasmid vectors. Subclones from phages containing markers C212(p322), C272(132SE11), C161(He3), AFM157xd10(131xb4) and CMS1(p11M1) were used as probes.

EXAMPLE 2

Pulsed field gel electrophoresis analysis

High molecular weight DNA was isolated in agarose plugs from Epstein-Barr virus transformed lymphoblastoid cell lines established from controls and patients or from YAC clone as described. Plugs were rinsed twice for 30 min. each in 10-20 min vol. TE. The plugs were equilibrated for 30' at 4°C with 0.3 ml of the appropriate restriction enzyme buffer containing 0.1 mg/ml BSA (Pharmacia). Excess buffer was then removed and the plugs were incubated at the appropriate temperature for 16 h with 40 U restriction enzyme per reaction. DNA was digested with the restriction enzymes BssHII, EagI, SfiI, SacI, KpnI,

SacII, SpeI. Separation of DNA fragments was performed using a CHEF-III-DR PFGE apparatus (Biorad). Fragments from 50 to 1200 kb were separated by electrophoresis through 1% agarose Seakem, at 200 V for 24 h at 14°C in 0.5 XTBE running buffer using a 30' to 70' ramping pulse time. The separation of fragments from 5 to 100 kb was performed by electrophoresis at 200 V for 19 h at 14°C in 0.5 x TBE buffer using a 5' to 20' ramping pulse time. After treatment with 0.25N HCl for 20 min, pulsed field gels were blotted onto Hybond N+ Nylon membrane (Amersham) in 0.4N NaOH, 0.4M NaCl for 20 h. Probes were successively hybridized to the same filters to ensure accurate data. Hybridizations were performed as described.

EXAMPLE 3

YAC library screening

YAC libraries from CEPH were screened by PCR with microsatellites C212, C272, C171, CMS1, and C161. YAC genotypes were established by electrophoresis of PCR products on denaturing polyacrylamide gels. YAC size was estimated by pulsed field gel electrophoresis.

EXAMPLE 4

Southern blot analysis

DNA samples were extracted from either peripheral blood leukocytes or lymphoblastoid cell lines. DNA were digested with restriction enzymes EcoRI, HindIII, BglII, XbaI, PvuII, XmnI, RsaI, PstI, BamHI, separated by electrophoresis on an 0.8% agarose gel for Southern blotting and hybridized with radioactively labeled probes.

EXAMPLE 5

Dinucleotide repeat polymorphisms

Genotypic data were obtained for the C212(D5F149S1, -S2), C272(D5F150S1, -S2) and C161(D5F153S1, -S2) dinucleotide repeat. Amplification conditions were as follows : denaturation at 94°C, annealing at 55°C, and extension at 72°C, 1 min each for 30 cycles. The procedure used for detection of dinucleotide repeat polymorphisms has been described elsewhere.

EXAMPLE 6

cDNA clone and DNA sequencing

Two million recombinants of a λgt10 human fetal brain library were plated according to the manufacturer (Clontech). Prehybridization and hybridization was carried out in 10% Dextran Sulphate Sodium, 1 M NaCl, 0.05 M Tris-HCl pH 7.5, 0.005 M EDTA and 1% SDS with 200 mg/ml sheared human placental DNA (Sigma) for 16 hours at 65°C. The filters were washed in 0.1X SSEP-0.1% SDS at 65°C and autoradiographs were performed for 24 hours. The DNA of positive cDNA clones were purified, digested with EcoRI and subcloned in M13 bacteriophage. Single strand DNAs were sequenced using the DyeDeoxy™ Terminator Cycle Sequencing Kit protocol supplied by Applied Biosystems, Inc. and analyzed on a ABI model 373A DNA automated sequencer. To obtain the 3' end of the cDNA along with its poly(A)$^+$ tail, PCR-amplification of a lymphoblastoid cell line cDNA library was performed using specific primer complementary to the 3' end of the clones and primer specific to the vectors arms of the cDNA library as previously described (Fournier B., Saudubray J.M., Benichou B. et al, 1994, J. Clin. Invest. 94:526-531). Specific PCR-products were directly sequenced with both primers using the DyeDeoxy™ Terminator Cycle Sequencing Kit protocol supplied by Applied Biosystems, Inc. and analyzed on a ABI model 373A DNA automated sequencer.

EXAMPLE 7

Isolation of RNA and Northern blot analysis

mRNA from lymphoblast cell lines of controls and SMA patients were isolated with the QuickPrep mRNA purification kit (Pharmacia) according to the supplier's procedure. Total RNA was prepared following the single-step RNA isolation method described by Chomczynski and Sacchi (Analytic Biochemistry, 162:156-159 (1987)). The total RNA preparation was treated with RQ1-DNAse (Promega) to remove any contaminating genomic DNA. Northern blots were made from mRNA and total RNA by electrophoresis through 1.5% seakem agarose gel containing methyl mercuric hydroxide and transferred to positively charged membrane in 20 X SSC and heated for 2 hours at 80°C. ^{32}P-radiolabeled DNA probes were synthesized by a random priming method according to the manufacturer (Boehringer), and hybridized in a solution containing 5 X SSEP, 1% SDS, 5 X Denhardt's for 16 hours at 65°C. The membranes were washed to a final stringency of 0.1 X SSEP, 0.1% SDS at 65°C for 10 min Autoradiography was at -80°C with intensifying screens and Kodak XAR films for 2 to 10 days. The amount of mRNA was normalized with a b-actine cDNA probe. The autoradiographs were scanned at 600 nm in computerized densitometer (Hoeffer Scientific Instruments, San Francisco). A Northern blot with polyA+ RNA from several huma tissues was purchased from Clontech.

EXAMPLE 8

Reverse transcriptase-based PCR amplification and sequencing

Each PCR amplification was carried out in a final volume of 20 ml on single-strand cDNAs synthesized from the random hexamers-primed reverse transcription (Promega). The PCR reactions included 2 picomoles of forward and reverse primers and 1 unit Taq polymerase in the reaction buffer recommended by Perkin Elmer/Cetus. Parameters for PCR amplification consisted in 1 min at 94°C, 1 min at 55°C and 1 min at 72°C for 30 cycles followed by a final extension

period of 10 min at 72°C. Parameters for PCR amplification consisted in 1 min at 94°C, 1 min at 55°C and 1 min at 72°C for 30 cycles followed by a final extension period of 10 min at 72°C. The PCR products were cut from acrylamide gel and eluted in 100 ml of TE buffer. The diluted fragments were reamplified with the same primers prior direct sequencing. The PCR amplification products were cut from acrylamide gel and eluted in 100 ml of TE buffer. The diluted fragments were reamplified prior to direct sequencing with both primers using the DyeDeoxy™ Terminator Cycle Sequencing Kit protocol supplied by Applied Biosystems, Inc. and analyzed on a ABI model 373A DNA automated sequencer. Six sets of primers along the cDNA sequence were used to amplify DNA products for sequence analysis.

EXAMPLE 9

Computer-assisted Analysis

Sequence homology analysis with both nucleotide and protein sequences from 541C were performed using FASTA and BLAST through the CITI2 French network (Dessen P., Fondrat C., Velencien C., Mugnoer C., 1990, CABIOS; 6:355-356).

EXAMPLE 10

SSCP Analysis

For single strand conformation polymorphism (SSCP) analysis, DNA from peripheral leukocytes (200 ng) was submitted to PCR amplification using unlabelled primers (20 µM) in 25 µl amplification mixture containing 200 µM dNTPs, 1 unit of Taq polymerase (Gibco-BRL) and 0,1 µl of a ^{32}P dCTP (10mCi/ml, NEN). Amplified DNA was mixed with an equal volume of formamide loaded dye (95% formamide, 20 mM EDTA, 0.05% bromophenol blue, 0.05% xylene cyanol). The samples (5µl) were denatured for 10 mn at 95°C and loaded

onto a polyacrylamide gel (Hydroling MED, Bioprobe) and electrophoresed at 4°C for 18 to 24 hours at 4W. Gels were transferred onto 3 MM Whatman paper, dried and autoradiographed with Kodak X-OMAT films for 24 hours. To amplify the DNA sequence containing the divergence of exon 7 oligonucleotides R111 (5' AGACTATCAACTTAATTTCTGATCA 3') and 541C770 (5' TAAGGAATGTGAGCACCTTCCTTC 3') were used. To amplify the DNA sequence containing the divergence of exon 8 oligonucleotides 541C960 (5' GTAATAACCAAATGCAATGTGAA 3') and 541C1120 (5' CTACAACACCCTTCTCACAG 3') were used.

EXAMPLE 11

Cloning of the human SMN gene

Total yeast DNA from YAC clone 595C11 was purified via the method of Sambrook et al *supra* and partially digested with restriction enzyme Sau3A. DNA in the 12-23 kD size range was excised after 0.5% sea plague GTG agarose gel electrophoresis and precipitated with ethanol after β-agarase digestion. After partial fill-in of the Sau3A site, DNA was subcloned at the partially filled XhoI site of bacteriophage FIXII (Stratagene).

The full-length BCD541 cDNA was used as a probe to screen the FIXII phage library under conditions set forth in Sambrook et al, *supra*.

These phages, named M-132, L-5 and L-13 spanned the entire SMN gene as confirmed by restriction mapping using HindIII, EcoRI and BglII (see, Fig. 9) and Southern blot analysis.

The phages were then sequenced as described in Example 8. Once the gene was sequenced, it was then cloned into a pUC18 vector and recombinantly reproduced in large quantities that were purified for further use.

EXAMPLE 12

Cloning of the mouse SMN gene

A mouse fetal cDNA library was screened using the coding sequence of the human SMN cDNA as a probe according to Sambrook et al, supra.

Two overlapping mouse cDNA clones were found that had the entire sequence of mouse SMN, as revealed by sequencing methods described in Example 8 after being cloned into a pUC18 vector and M13 vectors.

EXAMPLE 13

Transgenic mouse

Transgenic mice containing multiple normal SMN genes OR SMN genes lacking exon 7 are produced by the methods according to Lee et al, Neuron, 13: 978-988 (1994). The transgenic animals are then tested and selected for the overexpression of the SMN gene or SMN gene lacking exon 7 via Southern, and/or Northern blots using the probes described in the present invention or by screening with antibodies described in the present invention in a Western blot.

Transgenic mice containing abnormal SMN genes are obtained by homologous recombination methods using mutated SMN genes as described by Kühn et al, Science, 269: 1427-1429 (1995) and Bradley, Current Opinion in Biotechnology, 2: 823-829 (1991). The transgenic animals are then tested and selected for the overexpression of the SMN gene via Southern, and/or Northern blots using the probes described in the present invention or by screening with antibodies described in the present invention in a Western blot selected for the abnormal SMN gene.

EXAMPLE 14

Polyclonal antibodies

100 µg of a synthetic antigen having sequence :

N-terminal G G V P E Q E D S V L F R R G T C-terminal

was dissolved in buffer and emulsified with an equal volume of Freund's complete adjuvant. 0.5 ml of the emulsified synthetic antigen-adjuvant was injected intramuscularly into a rabbit. Five weeks later, the rabbit was boosted and 20-40 ml of blood was drawn 8 days after each booster injection. The serum was then tested for the presence of antigen using RIA.

Polyclonal antibodies were also prepared by the same methods using the following sunthetic antigens :

N-terminal S R S P G N K S D N I K P K C-terminal
F R Q N Q K E G R C S H S L N

EXAMPLE 15

Gene Therapy

Using the adenovirus construct described by Ragot et al, Nature, Vol. 361 (1993), the normal SMN gene was inserted therein and injected intramuscularly into a patient lacking this gene. The patient is monitored using SSCP analysis as described in Example 10 above.

While the invention has been described in terms of various preferred embodiments, the skilled artisan will appreciate that various modifications,

substitutions, omissions and changes may be made without departing from the spine thereof. Accordingly, it is intended that the scope of the present invention be limited solely by the scope of the following claims, including equivalents thereof.

Case	1	2	3	4	5	6	7	8	9	10	11	12*
Sex	m	f	m	m	m	m	m	m	m	f	f	f
Age of death	d8	d6	d1	d25	d11	d13	4m	>3y	>3y	d20	>9y	>16m
Fetal movements diminished	+	+	-	+	-	-	+	-	+	-	+	+
Hypotonia	+	+	+	+	+	+	+	+	+	+	+	+
Respiratory Involvement	+	+	+	+	+	+	+	-	+	+	-	-
Neurogenic (EMG)	?	+	+	+	+	+	nd	+	+	+	+	+
Muscle Atrophy (MB)	+	+	+	+	+	+	+	+	+	+	+	+
Contractures												
Hips	-	-	-	-	-	+	-	+	-	-	+	+
Knees	+	+	+	+	+	+	-	+	-	-	+	+
Ankles	+	-	-	+	-	-	-	+	+	+	+	+
Elbows	-	+	+	-	-	-	+	+	-	-	-	-
Wrists	-	-	+	-	+	+	+	+	-	+	-	-
Fingers	-	+	-	-	+	+	-	-	-	-	-	-
Associated Signs	facial micro	Ao.Co	-	-	-	-	fract.	-	facial micro	facial micro	facial micro	-
C212/C272 markers	+	+	del	del	+	del	+	+	unlink	+	+	+
SMN gene	del	del	del	del	del	del	+	+	+	+	+	+

Abbreviations: +, present; -, absent; Ao.Co, aortic coartation; Fract., bone fracture, Facial. microg, facial involvement with micrognathia; nd, not done.* Both the child and her father were affected.

Table 1

CLAIMS for the UNITED STATES

1. An isolated human survival motor neuron (SMN) protein.

2. An isolated mouse survival motor neuron (SMN) protein.

3. A human SMN gene T-BCD541 comprising a cDNA sequence of Figure 3.

4. A human SMN gene according to Claim 3, which comprises the following intronic sequences:

- for intron n° 6 :

5' AATTTTTAAATTTTTTGTAGAGACAGGGTCTCATTATGTTGCCCAGGGTG
GTGTCAAGCTCCAGGTCTCAAGTGATCCCCCTACCTCCGCCTCCCAAAGTTGT
GGGATTGTAGGCATGAGCCACTGCAAGAAAACCTTAACTGCAGCCTAATAATT
GTTTTCTTTGGGATAACTTTTAAAGTACATTAAAAGACTATCAACTTAATTTC
TGATCATATTTTGTTGAATAAAATAAGTAAAATGTCTTGTGAACAAAATGCTT
TTTAACATCCATATAAAGCTATCTATATATAGCTATCTATGTCTATATAGCTA
TTTTTTTTAACTTCCTTTTATTTTCCTTACAG 3'

- for intron n° 7 :

5' GTAAGTCTGCCAGCATTATGAAAGTGAATCTTACTTTTGTAAAACTTTAT
GGTTTGTGGAAAACAAATGTTTTTGAACAGTTAAAAAGTTCACATGTTAAAAA
GTTGAAAGGTTAATGTAAAACAATCAATATTAAAGAATTTTGATGCCAAAACT
ATTAGATAAAAGGTTAATCTACATCCCTACTAGAATTCTCATACTTAACTGGT
TGGTTATGTGGAAGAAACATACTTTCACAATAAAGAGCTTTAGGATATGATGC
CATTTTATATCACTAGTAGGCAGACCAGCAGACTTTTTTTTATTGTGATATGG
GATAACCTAGGCATACTGCACTGTACACTCTGACATATGAAGTGCTCTAGTCA
AGTTTAACTGGTGTCCACAGAGGACATGGTTTAACTGGAATTCGTCAAGCCTC
TGGTTCTAATTTCTCATTTGCAG 3'

5. The SMN gene according to Claim 3, which hybridizes in stringent conditions with the sequence of Figure 3 used as probe.

6. An isolated variant of the SMN gene, which variant is a C-BCD541 gene comprising a cDNA sequence of Figure 2.

7. An isolated nucleotide sequence comprising nucleotides 34 to 915 of the sequence of Figure 3.

8. An isolated nucleotide sequence comprising nucleotides 34 to 915 of the sequence of Figure 2.

9. An isolated DNA sequence encoding a survival motor neuron (SMN) protein of Figure 1 or Figure 8.

10. An isolated nucleotide sequence, comprising at least around 9 nucleotides within a sequence of Claim 3 or hybridizing in stringents conditions with a sequence of any one of Claims 1 to 9.

11. A mouse SMN gene comprising a cDNA sequence corresponding to the sequence of Figure 12.

12. A probe comprising the isolated nucleotide sequence of Claim 10.

13. A probe comprising the isolated nucleotide sequence of Claim 11.

14. An isolated nucleotide sequence selected among the following sequences :

5' AGACTATCAACTTAATTTCTGATCA 3'
5' TAAGGAATGTGAGCACCTTCCTTC 3'
5' GTAATAACCAAATGCAATGTGAA 3'
5' CTACAACACCCTTCTCACAG 3'

15. A set of primers comprising :

- a pair of primers contained in the sequence comprising nucleotides 921 to 1469 of the sequence of Figure 3 and/or

- a pair of primers comprising the following sequences :

5' AGACTATCAACTTAATTTCTGATCA 3'

5' TAAGGAATGTGAGCACCTTCCTTC 3'

16. A set of primers selected from the group consisting of :

5' AGACTATCAACTTAATTTCTGATCA 3'

5' TAAGGAATGTGAGCACCTTCCTTC 3' ;

5' GTAATAACCAAATGCAATGTGAA 3'

5' CTACAACACCCTTCTCACAG 3' ;

5' AGG GCG AGG CTC TGT CTC A 3'

5' CGG GAG GAC CGC TTG TAG T 3' ;

5' GCC GGA AGT CGT CAC TCT T 3'

5' GGG TGC TGA GAG CGC TAA TA 3' ;

5' TGT GTG GAT TAA GAT GAC TC 3'

5' CAC TTT ATC GTA TGT TAT C 3' ;

5' CTG TGC ACC ACC CTG TAA CAT G 3'

5' AAG GAC TAA TGA GAC ATC C 3' ;

5' CGA GAT GAT AGT TTG CCC TC 3'

5' AG CTA CTT CAC AGA TTG GGG AAA G 3' ;

5' CTC ATC TAG TCT CTG CTT CC 3'
5' TGG ATA TGG AAA TAG AGA GGG AGC 3' ;

5' CAC CCT TAT AAC AAA AAC CTG C 3'
5' GAG AAA GGA GTT CCA TGC AGC AG 3' ;

5' GAG AGG TTA AAT GTC CCG AC 3'
5' GTG AGA ACT CCA GGT CTC CTG G 3' ;

5' TGA GTC TGT TTG ACT TCA GG 3'
5' GAA GGA AAT GGA GGC AGC CAG C 3' ;

5' TTT CTA CCC ATT AGA ATC TGG 3'
5' CCC CAC TTA CTA TCA TGC TGG CTG 3' ;

5' CCA GAC TTT ACT TTT TGT TTA CTG 3'
5' ATA GCC ACT CAT GTA CCA TGA 3' ;

5' AAG AGT AAT TTA AGC CTC AGA CAG 3'
5' CTC CCA TAT GTC CAG ATT CTC TTG 3' ;

5' AGA CTA TCA ACT TAA TTT CTG ATC A 3'
5' TAA GGA ATG TGA GCA CCT TCC TTC 3' ;

5' AGA CTA TCA ACT TAA TTT CTG ATC A 3'
5' GTA AGA TTC ACT TTC ATA ATG CTG 3' ;

5' CTT TAT GGT TTG TGG AAA ACA 3'
5' GGC ATC ATA TCC TAA AGC TC 3' ;

5' CGA GAT GAT AGT TTG CCC TC 3'
5' AG CTA CTT CAC AGA TTG GGG AAA G 3'

5' CTC ATC TAG TCT CTG CTT CC 3'
5' TGG ATA TGG AAA TAG AGA GGG AGC 3'

5' CAC CCT TAT AAC AAA AAC CTG C 3'
5' GAG AAA GGA GTT CCA TGG AGC AG 3'

5' GAG AGG TTA AAT GTC CCG AC 3'
5' GTG AGA ACT CCA GGT CTC CTG G 3'

5' TGA GTC TGT TTG ACT TCA GG 3'
5' GAA GGA AAT GGA GGC AGC CAG C 3'

5' TTT CTA CCC ATT AGA ATC TGG 3'
5' CCC CAC TTA CTA TCA TGC TGG CTG 3'

5' CCA GAC TTT ACT TTT TGT TTA CTG 3'
5' ATA GCC ACT CAT GTA CCA TGA 3'

5' AAG AGT AAT TTA AGC CTC AGA CAG 3'
5' CTC CCA TAT GTC CAG ATT CTC TTG 3'

5' AGA CTA TCA ACT TAA TTT CTG ATC A 3'
5' TAA GGA ATG TGA GCA CCT TCC TTC 3'

5' AGA CTA TCA ACT TAA TTT CTG ATC A 3'
5' GTA AGA TTC ACT TTC ATA ATG CTG 3'

5' CTT TAT GGT TTG TGG AAA ACA 3'
5' GGC ATC ATA TCC TAA AGC TC 3'

5' GTA ATA ACC AAA TGC AAT GTG AA 3'
5' CTA CAA CAC CCT TCT CAC AG 3'

5' GGT GTC CAC AGA GGA CAT GG 3'
5' AAG AGT TAA CCC ATT CCA GCT TCC 3'

17. Antisense nucleotide sequence which is an invert complementary sequence of a sequence according to any one of Claims 1 to 11

18. An isolated human survival motor neuron (SMN) protein comprising the amino acid sequence of Figure 1.

19. A protein according to Claim 18, which is truncated and which comprises the sequence of Figure 8.

20. An isolated mouse survival motor neuron (SMN) protein comprising the amino acid sequence of Figure 12.

21. Kit for the in vitro detection of motor neuron diseases, comprising :
- a set of primers according to any one of Claims 15 or 16 ;
- reagents for an amplification reaction ; and
- a probe for the detection of the amplified product.

22. Kit for the in vitro detection of motor neuron diseases, comprising a probe according to Claim 12.

23. Kit according to Claim 21 or 22, for the detection of SMA.

24. Cloning or expression vector, characterized in that it comprises a sequence according to any one of Claims 1 to 11.

25. Vector according to Claim 24, characterized in that it has a motor neuron tropism.

26. Vector according to Claim 25, characterized in that it is for example a poliovirus, an adenovirus or a herpes virus.

27. Vector according to Claim 24, characterized in that it is a retrovirus vector.

28. Host cell, for example bone marrow cells, fibroblasts, epithelial cells, characterized in that it is transformed by a vector according to any one of Claims 24 to 27.

29. Recombinant nucleotide sequence, characterized in that it comprises a sequence of any one of Claims 1 to 11 and a sequence capable of encoding a polypeptide having a tropisme for the motor neuron.

30. A method for detecting motor neuron disorders including spinal muscular atrophy, any trophic lateral sclerosis and primary lateral sclerosis, said method comprising the steps of :

(a) extracting DNA from a patient sample;

(b) amplifying said DNA with primers according to any one of Claims 15 or 16;

(c) subjecting said amplified DNA to SCCP;

(d) autoradiographing the gels; and

(e) detecting the presence or absence of the motor neuron disorder.

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant: J. MELKI et al.

Serial No.: NEW Group: Unassigned

Filed: July 2, 1998 Examiner: Unassigned

For: SPINAL MUSCULAR ATROPHY DIAGNOSTIC METHODS (As Amended)

PRELIMINARY AMENDMENT

Assistant Commissioner for Patents
Washington, D.C. 20231

July 2, 1998

Sir:

The following preliminary amendments and remarks are respectfully submitted in connection with the above-identified application.

IN THE TITLE:

Please change the title to read:

--SPINAL MUSCULAR ATROPHY DIAGNOSTIC METHODS--.

IN THE SPECIFICATION:

Please insert before the claims in this application the Sequence Listing enclosed herewith. Please renumber the remaining pages, beginning with the claims, consecutively from page 84.

Please amend the specification as follows:

Page 7

Last Line, after "3'" insert --(SEQ ID NO:1)--

Page 8

Line 10, at the end of the line, insert --(SEQ ID NO:2)--

Line 18, at the end of the line, insert --(SEQ ID NO:3)--

Line 27, at the end of the line, insert --(SEQ ID NO:4)--

Page 9

Line 2, after "Figure 3" insert --(SEQ ID NOS:12-13)--

Line 6, after "Figure 2" insert --(SEQ ID NOS:10-11)--

Line 10, after "Figure 1" insert --(SEQ ID NO:9)--

Line 12, after "is" (first occurrence) insert --not--

Page 10

Line 9, at the end of the line, insert --(SEQ ID NO:1)--

Line 19, at the end of the line, insert --(SEQ ID NO:2)--

Line 27, at the end of the line, insert --(SEQ ID NO:3)--

Page 11

Line 9, at the end of the line, insert --(SEQ ID NO:4)--

Line 24, at the end of the line, insert --(SEQ ID NO:5)--

Line 25, at the end of the line, insert --(SEQ ID NO:6)--

Page 12

Line 2, at the end of the line, insert --(SEQ ID NO:7)--

Line 3, at the end of the line, insert --(SEQ ID NO:8)--

Line 13, at the end of the line, insert --(SEQ ID NO:5)--

Line 14, at the end of the line, insert --(SEQ ID NO:6)--

Line 17, at the end of the line, insert --(SEQ ID NO:5)--

Line 18, at the end of the line, insert --(SEQ ID NO:6)--

Line 20, at the end of the line, insert --(SEQ ID NO:7)--

Line 21, at the end of the line, insert --(SEQ ID NO:8)--

<u>Page 13</u>

Line 7, at the end of the line, insert --(SEQ ID NO:24)--

Line 8, at the end of the line, insert --(SEQ ID NO:25)--

Line 10, at the end of the line, insert --(SEQ ID NO:26)--

Line 11, at the end of the line, insert --(SEQ ID NO:27)--

Line 13, at the end of the line, insert --(SEQ ID NO:28)--

Line 14, at the end of the line, insert --(SEQ ID NO:29)--

Line 16, at the end of the line, insert --(SEQ ID NO:30)--

Line 17, at the end of the line, insert --(SEQ ID NO:31)--

Line 19, at the end of the line, insert --(SEQ ID NO:32)--

Line 20, at the end of the line, insert --(SEQ ID NO:33)--

Line 22, at the end of the line, insert --(SEQ ID NO:34)--

Line 23, at the end of the line, insert --(SEQ ID NO:35)--

<u>Page 14</u>

Line 2, at the end of the line, insert --(SEQ ID NO:36)--

Line 3, at the end of the line, insert --(SEQ ID NO:37)--

Line 5, at the end of the line, insert --(SEQ ID NO:38)--

Line 6, at the end of the line, insert --(SEQ ID NO:39)--

Line 8, at the end of the line, insert --(SEQ ID NO:40)--

Line 9, at the end of the line, insert --(SEQ ID NO:41)--

Line 11, at the end of the line, insert --(SEQ ID NO:42)--

Line 12, at the end of the line, insert --(SEQ ID NO:43)--

Line 14, at the end of the line, insert --(SEQ ID NO:44)--

Line 15, at the end of the line, insert --(SEQ ID NO:45)--

Line 17, at the end of the line, insert --(SEQ ID NO:46)--

Line 18, at the end of the line, insert --(SEQ ID NO:47)--

Line 20, at the end of the line, insert --(SEQ ID NO:48)--

Line 21, at the end of the line, insert --(SEQ ID NO:49)--

Line 23, at the end of the line, insert --(SEQ ID NO:50)--

Line 24, at the end of the line, insert --(SEQ ID NO:51)--

<u>Page 15</u>

Line 2, at the end of the line, insert --(SEQ ID NO:52)--

Line 3, at the end of the line, insert --(SEQ ID NO:53)--

Line 5, at the end of the line, insert --(SEQ ID NO:54)--

Line 6, at the end of the line, insert --(SEQ ID NO:55)--

Line 8, at the end of the line, insert --(SEQ ID NO:56)--

Line 9, at the end of the line, insert --(SEQ ID NO:57)--

<u>Page 17</u>

Line 23, after "Figure 1" insert --(SEQ ID NO:9)-- and after "Figure 8" insert --(SEQ ID NO:19)--

Line 24, after "Figure 12" insert --(SEQ ID NO:20)--

Page 18

Line 19, after "Fig. 1" insert --(SEQ ID NO:9)--

Line 21, change "Fig. 2 is th" to --Fig. 2A (SEQ ID NO:10) is the--

Page 19

Line 1, after "Fig. 2B" insert --(SEQ ID NO:11)--

Line 7, after "Fig. 3A" insert --(SEQ ID NO:12)--

Line 12, after "Fig. 3B" insert --(SEQ ID NO:13)--

Line 14, change "C212, C272, C171," to --C212 (SEQ ID NO:14), C272 (SEQ ID NO:15), C171 (SEQ ID NO:18)--

Line 15, after "AFM157xd10" insert --(SEQ ID NO:16)-- and after "C161" insert --(SEQ ID NO:17)--

Line 21, after "Fig. 8" insert --(SEQ ID NO:19)--

Page 20

Line 7, after "Fig. 10" insert --(SEQ ID NO:21)--

Line 15, after "Fig. 11" insert --(SEQ ID NO:22)--

Line 20, after "Fig. 12" insert --(SEQ ID NO:20)--

Page 22

Last Line, at the end of the line, insert --(SEQ ID NOS:14-18)--

Page 34

Line 25, before "C-terminal" insert --(residues 9-25 of SEQ ID NO:9)--

Line 26, at the end of the line, insert --(residues 173-186 of SEQ ID NO:9)--

Line 27, at the end of the line, insert --(residues 280-294 of SEQ ID NO:9)--

Page 45

Line 5, before "and" insert --(SEQ ID NO:5)--

Line 6, before "were" insert --(SEQ ID NO:6)--

Line 8, before "and" insert --(SEQ ID NO:7)--

Line 9, before "were" insert --(SEQ ID NO:8)--

Page 47

Line 4, before "C-terminal" insert --(residues 9-25 of SEQ ID NO:9)--

Line 12, before "C-terminal" insert --(residues 173-186 of SEQ ID NO:9)--

Line 13, at the end of the line, insert --(residues 280-294 of SEQ ID NO:9)--

IN THE CLAIMS:

Please amend the claims as follows:

Claim 4. (Amended) A human SMN gene according to Claim 3, which comprises the following intronic sequences:

-for intron n° 6 SEQ ID NO:1:

[5' AATTTTTAAATTTTTTGTAGAGACAGGGTCTCATTATGTTGCCCAGGGTG
GTGTCAAGCTCCAGGTCTCAAGTGATCCCCCTACCTCCGCCTCCCAAAGTTGT
GGGATTGTAGGCATGAGCCACTGCAAGAAAACCTTAACTGCAGCCTAATAATT
GTTTTCTTTGGGATAACTTTTAAAGTACATTAAAAGACTATCAACTTAATTTC
TGATCATATTTTGTTGAATAAAATAAGTAAAATGTCTTGTGAACAAAATGCTT
TTTAACATCCATATAAAGCTATCTATATATAGCTATCTATGTCTATATAGCTA
TTTTTTTTAACTTCCTTTTATTTTCCTTACAG 3']

-for intron n° 7 SEQ ID NO:2:

[5' GTAAGTCTGCCAGCATTATGAAAGTGAATCTTACTTTTGTAAAACTTTAT
GGTTTGTGGAAAACAAATGTTTTTGAACAGTTAAAAAGTTCAGATGTTAAAAA
GTTGAAAGGTTAATGTAAAACAATCAATATTAAAGAATTTTGATGCCAAAACT
ATTAGATAAAAGGTTAATCTACATCCCTACTAGAATTCTCATACTTAACTGGT
TGGTTATGTGGAAGAAACATACTTTCACAATAAAGAGCTTTAGGATATGATGC
CATTTTATATCACTAGTAGGCAGACCAGCAGACTTTTTTTATTGTGATATGG
GATAACCTAGGCATACTGCACTGTACACTCTGACATATGAAGTGCTCCTAGTCA
AGTTTAACTGGTGTCCACAGAGGACATGGTTTAACTGGAATTCGTCAAGCCTC
TGGTTCTAATTTCTCATTTGCAG 3'].

Claim 10, line 2, change "Claim 3" to --cDNA sequence of Fig. 3--;

change "in stringents" to --under stringent--;

line 3, change "Claims 1 to 9" to --Claims 3 to 9--.

Claim 14. (Amended) An isolated nucleotide sequence selected among the following sequences:

5' AGACTATCAACTTAATTTCTGATCA 3' (SEQ ID NO:5)

5' TAAGGAATGTGAGCACCTTCCTTC 3' (SEQ ID NO:6)

5' GTAATAACCAAATGCAATGTGAA 3' (SEQ ID NO:7)

5' CTACAACACCCTTCTCACAG 3' (SEQ ID NO:8)

Claim 15. (Amended) A set of primers comprising:

-a pair of primers contained in the sequence comprising nucleotides 921 to 1469 of the sequence of Figure 3 and/or

-a pair of primers comprising the following sequences:

5' AGACTATCAACTTAATTTCTGATCA 3' (SEQ ID NO:5)

5' TAAGGAATGTGAGCACCTTCCTTC 3' (SEQ ID NO:6).

Claim 16. (Amended) A set of primers selected from the group consisting of:

5' AGACTATCAACTTAATTTCTGATCA 3' (SEQ ID NO:5)

5' TAAGGAATGTGAGCACCTTCCTTC 3' (SEQ ID NO:6)

5' GTAATAACCAAATGCAATGTGAA 3' (SEQ ID NO:7)

5' CTACAACACCCTTCTCACAG 3' (SEQ ID NO:8)

5' AGG GCG AGG CTC TGT CTC A 3' (SEQ ID NO:24)

5' CGG GAG GAC CGC TTG TAG T 3' (SEQ ID NO:25);

5' GCC GGA AGT CGT CAC TCT T 3' (SEQ ID NO:26)

5' GGG TGC TGA GAG CGC TAA TA 3' (SEQ ID NO:27);

5' TGT GTG GAT TAA GAT GAC TC 3' (SEQ ID NO:28)

5' CAC TTT ATC GTA TGT TAT C 3' (SEQ ID NO:29);

5' CTG TGC ACC ACC CTG TAA CAT G 3' (SEQ ID NO:30)

5' AAG GAC TAA TGA GAC ATC C 3' (SEQ ID NO:31);

5' CGA GAT GAT AGT TTG CCC TC 3' (SEQ ID NO:32)

5' AG CTA CTT CAC AGA TTG GGG AAA G 3' (SEQ ID NO:33);

5' CTC ATC TAG TCT CTG CTT CC 3' (SEQ ID NO:34)

5' TGG ATA TGG AAA TAG AGA GGG AGC 3' (SEQ ID NO:35);

5' CAC CCT TAT AAC AAA AAC CTG C 3' (SEQ ID NO:36)

5' GAG AAA GGA GTT CCA TGG AGC AG 3' (SEQ ID NO:37);

5' GAG AGG TTA AAT GTC CCG AC 3' (SEQ ID NO:38)

5' GTG AGA ACT CCA GGT CTC CTG G 3' (SEQ ID NO:39);

5' TGA GTC TGT TTG ACT TCA GG 3' (SEQ ID NO:40)

5' GAA GGA AAT GGA GGC AGC CAG C 3' (SEQ ID NO:41);

5' TTT CTA CCC ATT AGA ATC TGG 3' (SEQ ID NO:42)

5' CCC CAC TTA CTA TCA TGC TGG CTG 3' (SEQ ID NO:43);

5' CCA GAC TTT ACT TTT TGT TTA CTG 3' (SEQ ID NO:44)

5' ATA GCC ACT CAT GTA CCA TGA 3' (SEQ ID NO:45);

5' AAG AGT AAT TTA AGC CTC AGA CAG 3' (SEQ ID NO:46)

5' CTC CCA TAT GTC CAG ATT CTC TTG 3' (SEQ ID NO:47);

5' AGA CTA TCA ACT TAA TTT CTG ATC A 3' (SEQ ID NO:48)

5' TAA GGA ATG TGA GCA CCT TCC TTC 3' (SEQ ID NO:49);

5' AGA CTA TCA ACT TAA TTT CTG ATC A 3' (SEQ ID NO:50)

5' GTA AGA TTC ACT TTC ATA ATG CTG 3' (SEQ ID NO:51);

5' CTT TAT GGT TTG TGG AAA ACA 3' (SEQ ID NO:52)

5' GGC ATC ATA TCC TAA AGC TC 3' (SEQ ID NO:53);

[5' CGA GAT GAT AGT TTG CCC TC 3'

5' AG CTA CTT CAC AGA TTG GGG AAA G 3';

5' CTC ATC TAG TGT CTG CTT CC 3'

5' TGG ATA TGG AAA TAG AGA GGG AGC 3';

5' CAC CCT TAT AAC AAA AAC CTG C 3'

5' GAG AAA GGA GTT CCA TGG AGC AG 3';

5' GAG AGG TTA AAT GTC CCG AC 3'

5' GTG AGA ACT CCA GGT CTC CTG G 3';

5' TGA GTC TGT TTG ACT TCA GG 3'

5' GAA GGA AAT GGA GGC AGC CAG C 3';

5' TTT CTA CCC ATT AGA ATC TGG 3'

5' CCC CAC TTA CTA TCA TGC TGG CTG 3';

5' CCA GAC TTT ACT TTT TGT TTA CTG 3'

5' ATA GCC ACT CAT GTA CCA TGA 3';

5' AAG AGT AAT TTA AGC CTC AGA CAG 3'

5' CTC CCA TAT GTC CAG ATT CTC TTG 3';

5' AGA CTA TCA ACT TAA TTT CTG ATC A 3'

5' TAA GGA ATG TGA GCA CCT TCC TTC 3';

5' AGA CTA TCA ACT TAA TTT CTG ATC A 3'

5' GTA AGA TTC ACT TTC ATA ATG CTG 3';

5' CTT TAT GGT TTG TGG AAA ACA 3'

5' GGC ATC ATA TCC TAA AGC TC 3';]

5' GTA ATA ACC AAA TGC AAT GTG AA 3' (SEQ ID NO:54)

5' CTA CAA CAC CCT TCT CAC AG 3' (SEQ ID NO:55); and

5' GGT GTC CAC AGA GGA CAT GG 3' (SEQ ID NO:56)

5' AAG AGT TAA CCC ATT CCA GCT TCC 3' (SEQ ID NO:57).

Claim 17, line 2, change "Claims 1 to 11" to --Claims 3 to 9 and 11--.

Claim 23, line 1, delete "21 or"

Claim 24, line 2, change "Claims 1 to 11" to --Claims 3 to 9 and 11--.

Claim 28, lines 2-3, change "any one of Claims 24 to 27" to --Claim 24--.

Claim 29, line 2, change "Claims 1 to 11" to --Claims 3 to 9 and 11--.

Please add the following new claims:

--31. The method of Claim 30, wherein said motor neuron disorder is spinal muscular atrophy.--

--32. The method of Claim 30, wherein steps (c) and (d) are replaced with a step of digestion with a Bsrl enzyme.--

--33. A method for detecting spinal muscular atrophy said method comprising the steps of:

(a) extracting DNA from a patient sample;

(b) hybridizing said DNA with a DNA probe comprising all or part of the DNA sequence of Figure 3 under stringent conditions; and

(c) detecting the hybrids possibly formed.--

--34. The method according to Claim 33, wherein said probe is radiolabeled.--

--35. A monoclonal antibody or a polyclonal antiserum directed against the SMN protein of Figure 1, or against the protein of Figure 8, or against the protein of Figure 12.--

--36. A method for detecting arthrogryposis multiplex congenita (AMC), said method comprising the steps of:

(a) extracting DNA from a patient sample;

(b) amplifying said DNA via PCR using unlabeled primers from exon 7 and exon 8 of the SMN gene;

(c) subjecting said amplified DNA to SCCP;

(d) autoradiographing the gels; and

(e) detecting the presence or absence of arthrogryposis multiplex congenita.--

--37. An isolated nucleotide sequence of Figure 11.--

--38. A transgenic mouse that only expresses the human SMN protein of Figure 1.--

--39. A transgenic mouse that expresses a mutated SMN protein of Figure 1.--

--40. A method of detecting the presence in a human patient of an altered SMN gene associated with spinal muscular atrophy, comprising

analyzing exon 7 or exon 8 of a gene identified as T-BCD541 in a biological sample derived from the patient, and

comparing said exon to the corresponding exon derived from T-BCD541 from normal human tissue;

wherein an alteration of either exon 7 or exon 8 in said patient sample with reference to said normal tissue is indicative of the presence of an altered SMN gene associated with spinal muscular atrophy in said patient.--

--41. The method of claim 40, wherein said analyzing comprises

determining whether T-BCD541 exon 7 is present or absent in the patient sample.--

--42. The method of claim 40, wherein said analyzing comprises

determining whether T-BCD541 exon 8 is present or absent in the patient sample.--

--43. The method of either of claim 40, wherein said analyzing includes amplifying all or part of the T-BCD541 gene.--

--44. The method of claim 43, wherein said analyzing comprises

amplifying a nucleotide fragment from said patient sample comprising exon 7 of the T-BCD541 gene,

amplifying a nucleotide fragment from said patient sample comprising exon 8 of the T-BCD541 gene, and

determining whether said exon 7 and said exon 8 are present or absent in said amplified fragments.--

--45. The method of claim 44, wherein said determining includes

subjecting said exon 7 comprising nucleotide fragment to restriction enzyme digestion,

subjecting said exon 8 comprising nucleotide fragment to restriction enzyme digestion, and

analyzing enzymatic digestion products produced by said enzymatic digestions,

wherein an alteration of either exon 7 or exon 8 with reference to normal tissue is evidenced by an altered restriction enzymatic digestion pattern in one or both of said exons.--

--46. The method of claim 43, wherein said amplifying is carried out using a polymerase chain reaction using a primer

selected from the group consisting of SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7 and SEQ ID NO:8.--

--47. The method of claim 40, wherein said analyzing comprises subjecting said patient T-BCD541 gene to restriction cleavage.--

--48. The method of claim 40, wherein said analyzing comprises subjecting said patient T-BCD541 gene to single strand conformation polymorphism analysis.

--49. The method of claim 40, wherein said biological sample is selected from the group consisting of blood, cerebral fluid, peripheral blood leukocytes, a lymphoblastoid cell line and muscle tissue.--

--50. A method of confirming a clinical diagnosis of arthrogryposis multiplex congenita in a patient, comprising

analyzing exon 7 or exon 8 of a gene identified as T-BCD541 in a biological sample derived from the patient, and

comparing said exon to the corresponding exon derived from T-BCD541 from normal human tissue;

wherein an alteration of either exon 7 or exon 8 in said patient sample with reference to said normal tissue is indicative of the presence of an altered SMN gene associated with arthrogryposis multiplex congenita in said patient.--

--51. The method of claim 50, wherein said analyzing comprises

amplifying a nucleotide fragment from said patient sample comprising exon 7 of the T-BCD541 gene,

amplifying a nucleotide fragment of said patient sample comprising exon 8 of the T-BCD541 gene, and

determining whether said exon 7 and said exon 8 are present or absent in said amplified nucleotide fragments.--

--52. The method of claim 51, wherein said determining includes

subjecting said exon 7 comprising nucleotide fragment to restriction enzyme digestion,

subjecting said exon 8 comprising nucleotide fragment to restriction enzyme digestion, and

analyzing enzymatic digestion products produced by said enzymatic digestions,

wherein an alteration of either exon 7 or exon 8 with reference to normal tissue is evidenced by an altered restriction enzymatic digestion pattern in one or both of said exons.--

REMARKS

Enclosed herewith in full compliance with 37 C.F.R. §1.821-1.825 is a Substitute Sequence Listing to be inserted into the

specification as indicated above. The Substitute Sequence Listing in no way introduces new matter into the specification.

Please transfer the sequence disk from parent file Serial No. 08/545,196, filed on October 19, 1995, to this file. The disk copy and paper copy of the sequence listing are identical except for word processing formatting.

Claims 31-52 are added by the present Amendment. New claims 31-39 find support as indicated in the Preliminary Amendment of October 19, 1995 on parent application Serial No. 08/595,196. These claims are presented in order to bring out an important feature of the Applicants' invention -- that the invention is based on the Applicants' discovery that patients with spinal muscular atrophy exhibit alterations in the telomeric version of a gene identified by the Applicants and termed "T-BCD541". Support for claims 40-52 is found in the specification, as discussed below.

Independent claim 40 brings out the feature that the method of the invention focuses on analyzing exon 7 and/or exon 8 of the T-BCD541 gene from a patient sample and determining whether one or both of these exons is altered in comparison to the corresponding exons present in normal human tissue. This aspect of the invention can be found described, at least, at page 6, paragraph 5 of the specification, where it is stated that the T-BCD541 gene is responsible for motor neuron diseases of the SMA type, since its alteration either by partial or total deletion, by mutation or other modification, is sufficient to lead to a pathological state.

Claims 41-42 further specify, as described above and elsewhere in the application, that the alteration is a deletion of either of exons 7 or 8.

Claims 43-45 are directed to specific methods for analyzing the T-BCD541 gene, which include amplifying the gene, and particularly exons 7 or 8 thereof, and further (claim 45) subjecting the amplification products to enzymatic digestion. Support for these methods can be found, at least, at page 17, second paragraph, as well as at page 28, first through third full paragraphs. Claims 46-49 are directed to specific features, including the use of specific primers, as set forth, at least, at page 11, last paragraph, through page 15, and the use of specific forms of analysis and tissue sources, as described, for example at page 16, last paragraph, through page 17, in Example 10, (pp. 44-45) and page 26, paragraphs 3-4, and page 36, first paragraph.

Claims 50-53 are directed to a related aspect of the present invention which involves the use of genetic analysis of T-BCD541 to confirm a clinical diagnosis of arthrogryposis multiplex congenita (AMC). This feature embodies a further discovery of the present invention -- that AMC of neurogenic origin is related to SMA. Support for this aspect of the invention is provided, at least example, at page 38, second and third paragraphs and generally, at pages 36-38.

Favorable action on the merits is respectfully requested.

Should there be any outstanding matters which need to be resolved in the present application, the Examiner is respectfully

requested to contact Maryanne Liotta (Registration No. 40,069) at the telephone number of the undersigned below, to conduct an interview in an effort to expedite prosecution in connection with the present application.

If necessary, the Commissioner is hereby authorized in this, concurrent, and further replies, to charge payment or credit any overpayment to Deposit Account No. 02-2448 for any additional fee required under 37 C.F.R. §§ 1.16 or 1.17; particularly, extension of time fees.

Respectfully submitted,

BIRCH, STEWART, KOLASCH & BIRCH, LLP

By: Andrew Miller #32,868
for
C. Joseph Faraci
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CJF:MAL:jul
Attachment: Sequence Listing

MAMSSGGSGGGVPEQEDSVLFRRGTGQSDDSDIWDDTALIKAYDKAVAS
FKHALKNGDICETSGKPKTTPKRKPAKKNKSQKKNTAASLQQWKVGDKCSAIWSEDGCIY
PATIASIDFKRETCVVVYTGYGNREEQNLSDLLSPICEVANNIEQNAQENENESQVSTDE
SENSRSPGNKSDNIKPKSAPWNSFLPPPPPMPGPRLGPGKPGLKFNGPPPPPPPPPPHLL
SCWLPPFPSGPPIIPPPPPICPDSLDDADALGSMLISWYMSGYHTGYYMGFRQNQKEGRC
SHSLN

FIGURE 1

CGGGGCCCCACGCTGCGCACCCGCGGGTTTGCTATGGCGATGAGCAGCGGCGGCAGTGGT
GGCGGCGTCCCGGAGCAGGAGGATTCCGTGCTGTTCCGGCGCGGCACAGGCCAGAGCGAT
GATTCTGACATTTGGGATGATACAGCACTGATAAAAGCATATGATAAAGCTGTGGCTTCA
TTTAAGCATGCTCTAAAGAATGGTGACATTTGTGAAACTTCGGGTAAACCAAAAACCACA
CCTAAAAGAAAACCTGCTAAGAAGAATAAAAGCCAAAAGAAGAATACTGCAGCTTCCTTA
CAACAGTGGAAAGTTGGGGACAAATGTTCTGCCATTTGGTCAGAAGACGGTTGCATTTAC
CCAGCTACCATTGCTTCAATTGATTTTAAGAGAGAAACCTGTGTTGTGGTTTACACTGGA
TATGGAAATAGAGAGGAGCAAAATCTGTCCGATCTACTTTCCCCAATCTGTGAAGTAGCT
AATAATATAGAACAGAATGCTCAAGAGAATGAAAATGAAAGCCAAGTTTCAACAGATGAA
AGTGAGAACTCCAGGTCTCCTGGAAATAAATCAGATAACATCAAGCCCAAATCTGCTCCA
TGGAACCCCTTTCTCCCTCCACCACCCCCCATGCCAGGGCCAAGACTGGGACCAGCAAAG
CCAGGTCTAAAATTCAATGGCCCACCACCGCCACCGCCACCACCACCACCCCACTTACTA
TCATGCTGGCTGCCTCCATTTCCTTCTGGACCACCAATAATTCCCCCACCACCTCCCATA
TGTCCAGATTCTCTTGATGATGCTGATGCTTTGGGAAGTATGTTAATTTCATGGTACATG
AGTGGCTATCATACTGGCTATTATATGGGTTTTAGACAAAATCAAAAAGAAGGAAGGTGC
TCACATTCCTTAAATTAAGGAGAAATGCTGGCATAGAGCAGCACTAAATGACACCACTAA
AGAAACGATCAGACAGATCTGGAATGTGAAGCGTTATAGAAGATAACTGGCCTCATTTCT
TCAAAATATCAACTGTTGGGAAAGAAAAAAGGAAGTGGAATGGGTAACTCTTCTTGATTA
AAAGTTATGTAATAACCAAATGCAATGTGAAATATTTTACTGGACTCTTTTGAAAAACCA
TCTGTAAAAGACTGAGGTGGGGGTGGGAGGCCAGCACGGTGGTGAGGCAGTTGAGAAAAT
TTGAATGTGGATTAGATTTTGAATGATATTGGATAATTATTGGTAATTTTATGGCCTGTG
AGAAGGGTGTTGTAGTTTATAAAAGACTGTCTTAATTTGCATACTTAAGCATTTAGGAAT
GAAGTGTTAGAGTGTCTTAAAATGTTTCAAATGGTTTAACAAAATGTATGTGAGGCGTAT
GTGGCAAAATGTTACAGAATCTAACTGGTGGACATGGCTGTTCATTGTACTGTTTTTTTC
TATCTTCTATATGTTTAAAAGTATATAATAAAAATATTTAATTTTTTTTTAAAAAAAAAA
AAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA
AAAAAAAAAAAAAAAAAAAAAAA

FIGURE 2A

AATTTTTAAATTTTTTGTAGAGACAGGGTCTCATTATGTTGCCCAGGGTGGTGTCAAGCTCCA
GGTCTCAAGTGATCCCCCTACCTCCGCCTCCCAAAGTTGTGGATTGTAGGCATGAGCCACTG
CAAGAAAACCTTAACTGCAGCCTAATAATTGTTTTCTTTGGGATAACTTTTAAAGTACATTAA
AAGACTATCAACTTAATTTCTGATCATATTTTGTTGAATAAAATAAGTAAAATGTCTTGTGAA
CAAAATGCTTTTTAACATCCATATAAAGCTATCTATATATAGCTATCTATATCTATATAGCTA
TTTTTTTTAACTTCCTTTTATTTTCCTTACAG*GGTTTCAGACAAAATCAAAAAGAAGGAAGG
TGCTCACATTCCTTAAATTAAGGA*GTAAGTCTGCCAGCATTATGAAAGTGAATCTTACTTTT
GTAAAACTTTATGGTTTGTGGAAAACAAATGTTTTTGAACAGTTAAAAAGTTCAGATGTTAGA
AAGTTGAAAGCTTAATGTAAAACAATCAATATTAAAGAATTTTGATGCCAAAACTATTAGATA
AAAGGTTAATCTACATCCCTACTAGAATTCTCATACTTAACTGGTTGGTTGTGTGGAAGAAAC
ATACTTTCACAATAAAGAGCTTTAGGATATGATGCCATTTTATATCACTAGTAGGCAGACCAG
CAGACTTTTTTTTATTGTGATATGGGATAACCTAGGCATACTGCACTGTACACTCTGACATAT
GAAGTGCTCTACTCAAGTTTAACTGGTGTCCACAGAGGACATGGTTTAACTGGAATTCGTCAA
GCCTCTGGTTCTAATTTCTCATTTGCAG*GAAATGCTGGCATAGAGCAGCACTAAATGACACC
ACTAAAGAAACGATCAGACACATCTGGAATGTGAAGCGTTATAGAAGATAACTGGCCTCATTT
CTTCAAAATATCAAGTGTTGGGAAAGAAAAAAGGAAGTGGAATGGGTAACTCTTCTTGATTA
AAAGTTATGTAATAACCAAATGCAATGTGAAATATTTTACTGGACTCTTTTGAAAAAC
CATCTGTAAAAGACTGGGGTGGGGGTGGGAGGCCAGCACGGTGGTGAGGCAGTTGAGAAAA
TTTGAATGTGGATTAGATTTTGAATGATATTGGATAATTATTGGTAATTTTATGGCCTGT
GAGAAGGGTGTTGTAGTTTATAAAAGACTGTCTTAATTTGCATACTTAAGCATTTAGG
AATGAAGTGTTAGAGTGTCTTAAAATGTTTCAAATGGTTTAACAAAATGTATGTGAGGCGT
ATGTGGCAAAATGTTACAGAATCTAACTGGTGGACATGGCTGTTCATTGTACTGTTTTTT
TCTATCTTCTATATGTTTAAAAGTATATAATAAAAATATTTAATTT

FIGURE 2B

1
CGGGGCCCCACGCTGCGCATCCGCGGGTTTGCTATGGCGATGAGCAGCGGCGGCAGTGGT
2
GGCGGCGTCCCGGAGCAGGAGGATTCCGTGCTGTTCCGGCGCGGCACAGGCCAG*ACGGAT
GATTCTGACATTTGGGATGATACAGCACTGATAAAAGCATATGATAAAGCTGTGGCTTCA
TTTAAGCATGCTCTAAAGAATGGTGACATTTGTGAAACTTCGGGTAAACCAAAAACCACA
CCTAAAAGAAAACCTGCTAAGAAGAATAAAAGCCAAAAGAAGAATACTGCAGCTTCCTTA
3
CAACAG*TGGAAAGTTGGGGACAAATGTTCTGCCATTTGGTCAGAAGACGGTTGCATTTAC
CCAGCTACCATTGCTTCAATTGATTTTAAGAGAGAAACCTGTGTTGTGGTTTACACTGGA
TATGGAAATAGAGAGGAGCAAAATCTGTCCGATCTACTTTCCCCAATCTGTGAAGTAGCT
4
AATAATATAGAACAGAATGCTCAAGAG*AATGAAAATGAAAGCCAAGTTTCAACAGATGAA
AGTGAGAACTCCAGGTCTCCTGGAAATAAATCAGATAACATCAAGCCCAAATCTGCTCCA
TGGAACTCTTTTCTCCCTCCACCACCCCCCATGCCAGGGCCAAGACTGGGACCAGGAAAG
5
*CCAGGTCTAAAATTCAATGGCCCACCACCGCCACCGCCACCACCACCACCCCACTTACTA
6
TCATGCTGGCTGCCTCCATTTCCTTCTGGACCACCA*ATAATTCCCCCACCACCTCCCATA
TGTCCAGATTCTCTTGATGATGCTGATGCTTTGGGAAGTATGTTAATTTCATGGTACATG
7
AGTGGCTATCATACTGGCTATTATATG*GGTTTCAGACAAAATCAAAAAGAAGGAAGGTGC
8
TCACATTCCTTAAATTAAGGA*GAAATGCTGGCATAGAGCAGCACTAAATGACACCACTAA
AGAAACGATCAGACAGATCTGGAATGTGAAGCGTTATAGAAGATAACTGGCCTCATTTCT
TCAAAATATCAAGTGTTGGGAAAGAAAAAAGGAAGTGGAATGGGTAACTCTTCTTGATTA
AAAGTTATGTAATAACCAAATGCAATGTGAAATATTTTACTGGACTCTTTTGAAAAAC
CATCTGTAAAAGACTGGGGTGGGGGTGGGAGGCCAGCACGGTGGTGAGGCAGTTGAGAAAA
TTTGAATGTGGATTAGATTTTGAATGATATTGGATAATTATTGGTAATTTTATGGCCTGT
GAGAAGGGTGTTGTAGTTTATAAAAGACTGTCTTAATTTGCATACTTAAGCATTTAGG
AATGAAGTGTTAGAGTGTCTTAAAATGTTTCAAATGGTTTAACAAAATGTATGTGAGGCGT
ATGTGGCAAAATGTTACAGAATCTAACTGGTGGACATGGCTGTTCATTGTACTGTTTTTT
TCTATCTTCTATATGTTTAAAAGTATATAATAAAAATATTTAATTTTTTTTTAAAAAAAA
AAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA

FIGURE 3A

AATTTTTAAATTTTTTGTAGAGACAGGGTCTCATTATGTTGCCCAGGGTGGTGTCAAGCTCCA
GGTCTCAAGTGATCCCCCTACCTCCGCCTCCCAAAGTTGTGGGATTGTAGGCATGAGCCACTG
CAAGAAAACCTTAACTGCAGCCTAATAATTGTTTTCTTTGGGATAACTTTTAAAGTACATTAA
AAGACTATCAACTTAATTTCTGATCATATTTTGTTGAATAAAATAAGTAAAATGTCTTGTGAA
CAAAATGCTTTTTAACATCCATATAAAGCTATCTATATATAGCTATCTATGTCTATATAGCTA
TTTTTTTTAACTTCCTTTTATTTTCCTTACAG*GGTTTCAGACAAAATCAAAAAGAAGGAAGG
TGCTCACATTCCTTAAATTAAGGA*GTAAGTCTGCCAGCATTATGAAAGTGAATCTTACTTTT
GTAAAACTTTATGGTTTGTGGAAAACAAATGTTTTTGAACAGTTAAAAAGTTCAGATGTTAAA
AAGTTGAAAGGTTAATGTAAAACAATCAATATTAAAGAATTTTGATGCCAAAACTATTAGATA
AAAGGTTAATCTACATCCCTACTAGAATTCTCATACTTAACTGGTTGGTTATGTGGAAGAAAC
ATACTTTCACAATAAAGAGCTTTAGGATATGATGCCATTTTATATCACTAGTAGGCAGACCAG
CAGACTTTTTTTTATTGTGATATGGGATAACCTAGGCATACTGCACTGTACACTCTGACATAT
GAAGTGCTCTAGTCAAGTTTAACTGGTGTCCACAGAGGACATGGTTTAACTGGAATTCGTCAA
GCCTCTGGTTCTAATTTCTCATTTGCAG*GAAATGCTGGCATAGAGCAGCACTAAATGACACC
ACTAAACAAACGATCAGACAGATCTGGAATGTGAAGCGTTATAGAAGATAACTGGCCTCATTT
CTTCAAAATATCAAGTGTTGGGAAAGAAAAAAGGAAGTGGAATGGGTAACTCTTCTTGATTA
AAAGTTATGTAATAACCAAATGCAATGTGAAATATTTTACTGGACTCTTTTGAAAAAC
CATCTGTAAAAGACTGGGGTGGGGGTGGGAGGCCAGCACGGTGGTGAGGCAGTTGAGAAAA
TTTGAATGTGGATTAGATTTTGAATGATATTGGATAATTATTGGTAATTTTATGGCCTGT
GAGAAGGGTGTTGTAGTTTATAAAAGACTGTCTTAATTTGCATACTTAAGCATTTAGG
AATGAAGTGTTAGAGTGTCTTAAAATGTTTCAAATGGTTTAACAAAATGTATGTGAGGCGT
ATGTGGCAAAATGTTACAGAATCTAACTGGTGGACATGGCTGTTCATTGTACTGTTTTTT
TCTATCTTCTATATGTTTAAAAGTATATAATAAAAATATTTAATTT

FIGURE 3B

C212

ACCTGANCCCAGANGGTCAAGGCTGCAGTGAGACGAGATTGCNCCACTGCCCTCC
ACCCTGGGTCATAAGAGTGGGACCCTGTNTCAAAACATACACACACACACACACA
CACACACACACACACACACACACACTCTCTCTCTCTCTCTCTCTCTCTCTC
TCTCTCTCTCTCTCAAAAACACTTGGTCTGTTATTTTTNCGAAATTGTCAGTCAT
AGTTATCTGTTAGACCAAAGCTGNGTAAGNACATTTATTACATTGCCTCCTACAA
CTTCATCAGCTAATGTATTTGCTATATAGCAATTACATATNGGNATATATTATCT
TNAGGGCATGGCCANGTNATAAAACTGTCACTGAGGAAAGGA

C272

CCTCCCACCTNAGCCTCCCCAGTAGCTAGGACTATAGGCGTGCNCCACCAAGCTC
AGCTATTTTTNNTATTTAGTAGAGACGGGGTTTCGGCANGCTTAGGCCTCGTNTC
GAACTCCAGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTG
TGTGTAGATATTTATTCCCCCTCCCCCTTGGAAAAGTAAGTAAGCTCCTACTAGG
AATTTAAAACCTGCTTGATCTATATAAAGACAAACAAGGAAAGACAAACATGGGG
GCAGGAAGGAAGGCAGATC

AFM157xd10

TCGAGGTAGATTTGTATTATATCCCATGTACACACACACACACACACACACACAC
ACACACACACACAGACTTAATCTGTTTACAGAAATAAAAGGAATAAAATACCGTT
TCTACTATACACCAAAACTAGCCATCTTGAC

C161

CCCTGAGAAGGCTTCCTCCTGAGTATGCATAAACATTCACAGCTTGCATGCGTGT
GTGTGTGTGTGTGTGTGTGTATGTTTGCTTGCACTGTAAAAACAATTGCAACATC
AACAGAAATAAAAATTAAAGGAATAATTCTCCTCCGACTCTGCCGTTCCATCCAG
TGAAACTCTTCATTCTGGGGTAAAGTTCCTTCAGTTCTTTCATAGATAGGTATAT
ACTTCATAAGTCAAACAATCAGGCTGGGTGCAGTAGCTCATGCCTGTAATCCCAG
CCCTTTGGGAGGCCGAGCTGGGCAGATCGA

C171

TCCACCCGCCTTGGCCTCCCAAAGCNCTGGGATTACAGGCGTGACTGCCGCACCC
AGCTGTAAACTGGNTTNNTAATGGTAGATTTTNAGGTATTAACAATAGATAAAAA
GATACTTTTNGGCATACTGTGTATTGGGATGGGGTTAGAACAGGTGTNCTACCCA
AGACATTTACTTAAAATCGCCCTCGAAATGCTATGTGAGCTGTGTGTGTGTGTGT
GTGTGTGTGTGTATTAAGGAAAAGCATGAAAGTATTTATGCTTGATTTTTTTTTT
TNACTCATACCTTCATAGTGGANCAGATACATAGTCTAAATCAAAATGTTTAAAC
TTTTTATGTCACTTGCTGTC

FIGURE 4

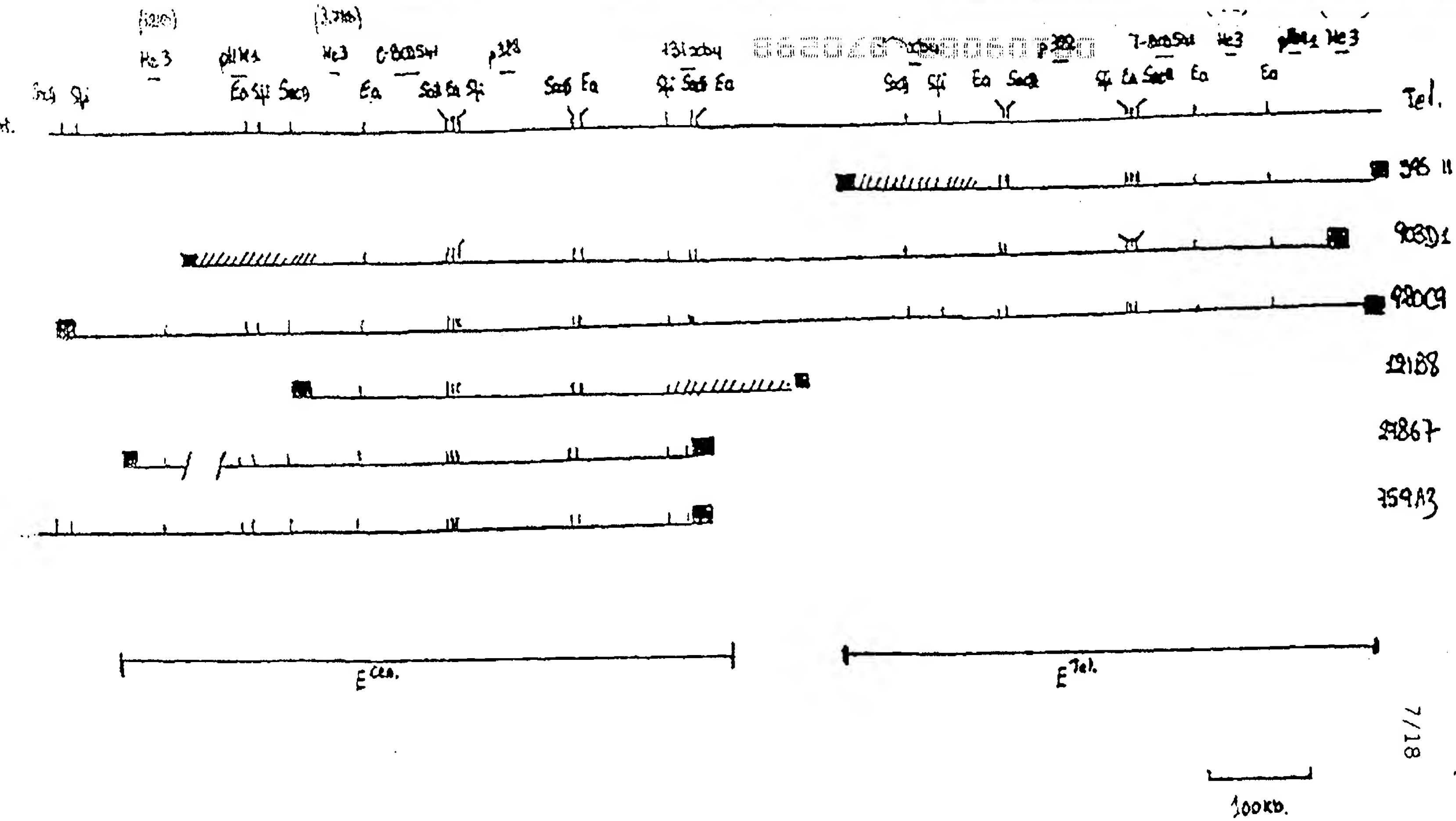
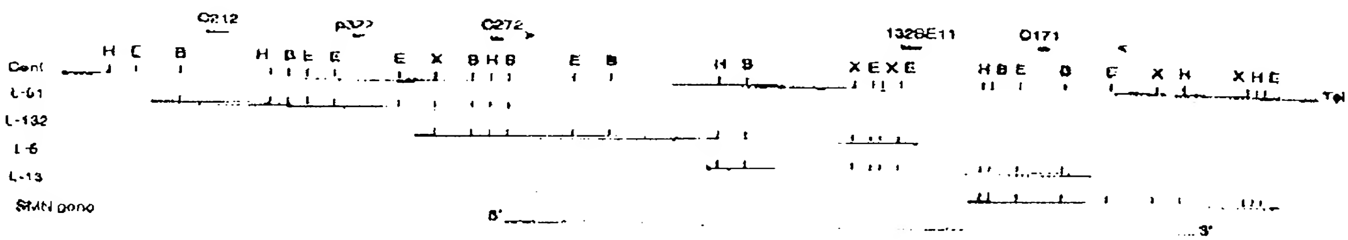


FIGURE 5

Restriction map of the 5q13 region. for EagI (Ea), SacII (SacII), SfiI (Sfi). Numbers under parenthesis indicate the restriction fragment detected by He3; Telomeric element (E^{Tel}), Centromeric element ($E^{Cen.}$) Centromere (Cent.), Telomere (Tel.). Probes are indicated above the restriction map. YACs are below the restriction map.



Telomeric element (E^{Tel}) containing the survival motor-neuron gene (SMN gene). Genetic map shows polymorphic markers C212, C272 and C171. Physical map shows location and direction of transcription of SMN gene; phage clones used for assembling physical map. Restriction map for EcoRI(E), XbaI(X), HindIII(H), BglII(B), SacII(S) are shown. Cent. and Tel. indicate centromere and telomere respectively. The position of genomic rearrangements found in SMA patients are also indicated.

FIGURE 6

Gene dosage analysis of the 5q13 region with the 132SE11 plasmid cone in SMA type I patient. Total human DNA from SMA family was digested with HindIII for Southern blotting. Filter was consecutively hybridized with 132SE11 (A) and JK53 probes (B). A significant decrease in 132SE11 band intensity, which indicated the deletion, compared with their parents. F/Father, M/Mother, A/affected

FIGURE 7

MAMSSGGSGGGVPEQEDSVLFRRGTGQSDDSDIWDDTALIKAYDKAVASFKHA
LKNGDICETSGKPKTTPKRKPAKKNKSQKKNTAASLQQWKVGDKCSAIWSEDG
CIYPATIASIDFKRETCVVVYTGYGNREEQNLSDLLSPICEVANNIEQNAQEN
ENESQVSTDESENSRSPGNKSDNIKPKSAPWNSFLPPPPPMPGPRLGPGKPGL
KFNGPPPPPPPPPPHLLSCWLPPFPSGPPIIPPPPPICPDSLDDADALGSMLI
SWYMSGYHTGYYM

FIGURE 8

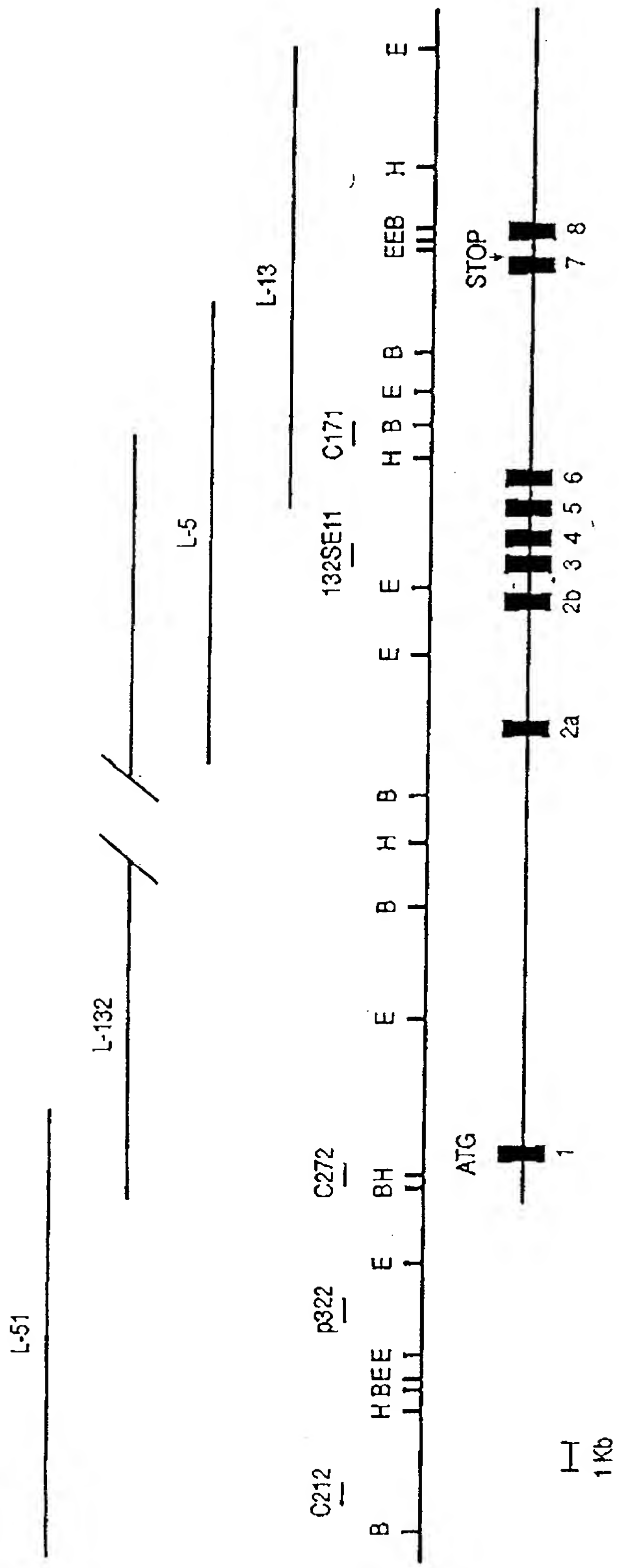

L-13
L-5
L-132
L-51
C171
132SE11
C272
p322
C212
STOP
ATG
1 Kb
2a
2b
3 4 5 6
7 8
1

Figure 9

```
1
cctcccgggcaccgtactgttccgctcccagaagccccgggcgccggaagtcgtcactcttaagaagggacg
gggccccacgctgcgcacccgcgggtttgct ATG GCG ATG AGC AGC GGC GGC AGT GGT GGC
                                 M   A   M   S   S   G   G   S   G   G
GGC GTC CCG GAG CAG GAG GAT TCC GTG CTG TTC CGG CGC GGC ACA GGC CAG  gtg
 G   V   P   E   Q   E   D   S   V   L   F   R   R   G   T   G   Q
aggtcgcagccagtgcagtctccctattagcgctctcagcacccttcttccggcccaactctccttccgca
2a
gtgtaatttgttatgtgtggattaagatgactcttggtactaacatacatttctgattaaacctatctgn
acatgagttgttttatttcttaccctttccag AGC GAT GAT TCT GAC ATT TGG GAT GAT
                                  S   D   D   S   D   I   W   D   D
ACA GCA CTG ATA AAA GCA TAT GAT AAA GCT GTG GCT TCA TTT AAG  gtatgaaatgc
 T   A   L   I   K   A   Y   D   K   A   V   A   S   F   K
ttgnttagtcgttttcttatttttcgttattcatttggaaaggaattgataacatacgataaagtgttaa
2b
aggtgctttctgaggtgacggagccttgagactagcttatagtagtaactgggttatgtcgtgacttttatt
ctgtgcaccaccctgtaacatgtacatttttattcctattttcgtag CAT GCT CTA AAG AAT GGT
                                                 H   A   L   K   N   G
GAC ATT TGT GAA ACT TCG GGT AAA CCA AAA ACC ACA CCT AAA AGA AAA CCT GCT
 D   I   C   E   T   S   G   K   P   K   T   T   P   K   R   K   P   A
AAG AAG AAT AAA AGC CAA AAG AAG AAT ACT GCA GCT TCC TTA CAA CAG  gttattt
 K   K   N   K   S   Q   K   K   N   T   A   A   S   L   Q   Q
taaaatgttgaggatttaacttcaaaggatgtctcattagtccttatttaatagtgtaaaatgtctttaact
3
gcctgcaggtcgatcaaaacgagatgatagtttgccctcttcaaaagaaatgtgtgcatgtatatatctttg
atttcttttgtag TGG AAA GTT GGG GAC AAA TGT TCT GCC ATT TGG TCA GAA GAC
               W   K   V   G   D   K   C   S   A   I   W   S   E   D
GGT TGC ATT TAC CCA GCT ACC ATT GCT TCA ATT GAT TTT AAG AGA GAA ACC TGT
 G   C   I   Y   P   A   T   I   A   S   I   D   F   K   R   E   T   C
GTT GTG GTT TAC ACT GGA TAT GGA AAT AGA GAG GAG CAA AAT CTG TCC GAT CTA
 V   V   V   Y   T   G   Y   G   N   R   E   E   Q   N   L   S   D   L
CTT TCC CCA ATC TGT GAA GTA GCT AAT AAT ATA GAA CAG AAT GCT CAA GAG  gta
 L   S   P   I   C   E   V   A   N   N   I   E   Q   N   A   Q   E
aggatacaaaaaaaaaaaattcaatttctggaagcagagactagatgagaaactgttaaacagtatacaca
4
ccaccgaggcattaatttttttcttaatcacacccttataacaaaaacctgcatatttttttctttttaaag
AAT GAA AAT GAA AGC CAA GTT TCA ACA GAT GAA AGT GAG AAC TCC AGG TCT CCT
 N   E   N   E   S   Q   V   S   T   D   E   S   E   N   S   R   S   P
GGA AAT AAA TCA GAT AAC ATC AAG CCC AAA TCT GCT CCA TGG AAC TCT TTT CTC
 G   N   K   S   D   N   I   K   P   K   S   A   P   W   N   S   F   L
CCT CCA CCA CCC CCC ATG CCA GGG CCA AGA CTG GGA CCA GGA AAG  gtaaaccttct
 P   P   P   P   P   M   P   G   P   R   L   G   P   G   K
atgaaagttttccagaaaatagttaatgtcgggacatttaacctctctgttaactaatttgtagctctccca
5
caaatattctgggtaattatttttatcctttttggttttgagtcctttttattcctatcatattgaaattggt
aagttaattttcctttgaaatattccttatag CCA GGT CTA AAA TTC AAT GGC CCA CCA CCG
                                  P   G   L   K   F   N   G   P   P   P
CCA CCG CCA CCA CCA CCA CCC CAC TTA CTA TCA TGC TGG CTG CCT CCA TTT CCT
 P   P   P   P   P   P   P   H   L   L   S   C   W   L   P   P   F   P
TCT GGA CCA CCA  gtaagtaaaaagagtataggttagattttgctttcacatacaattgataatta
 S   G   P   P
6
ccagactttactttttgtttactggatataaacaatatctttttctgtctccag ATA ATT CCC CCA
                                                        I   I   P   P
CCA CCT CCC ATA TGT CCA GAT TCT CTT GAT GAT GCT GAT GCT TTG GGA AGT ATG
 P   P   P   I   C   P   D   S   L   D   D   A   D   A   L   G   S   M
TTA ATT TCA TGG TAC ATG AGT GGC TAT CAT ACT GGC TAT TAT ATG  gtaagtaatca
 L   I   S   W   Y   M   S   G   Y   H   T   G   Y   Y   M
ctcagcatcttttcctgacaatttttttgtagttatgtgactttgtttggtaaatttataaaatactacttg
```

Figure 10

7
aactgcagcctaataattgttttctttgggataacttttaaagtacattaaaagactatcaacttaatttct
gatcatatttgttgaataaaataagtaaaatgtcttgtgaaacaaaatgcttttaacatccatataaagc

→ **a**

tatctatatatatagctatctatgtctatatatagtattttttttaacttcatttcattttttccttacag GGT

→ **T** G

TTC AGA CAA AAT CAA AAA GAA GGA AGG TGC TCA CAT TCC TTA AAT taaggagtaag
F R Q N Q K E G R C S H S L N *
tctgccagcattatgaaagtgaatcttactttgtaaactttatggtttgtggaaaacaaatgttttgaa

→ **g**

cagttaaaaagttcagatgttaaaaagttgaaaggttaatgtaaaacaatcaatattaaagaattttgatgc

→ **g**

caaaactattagataaaggttaatctacatccctactagaattctcatacttaactggttggttatgtgga
agaaacatactttcacaataaagagctttaggatatgatgccattttatatcactagtaggcagaccagcag
acttttttttattgtgatatgggataacctaggcatactgcactgtacactctgacatatgaagtgctctag
tcaagtttaactggtgtccacagaggacatggtttaactggaattcgtcaagcctctggttctaatttctca

8
tttgcaggaaatgctggcatagagcagcactaaatgacaccactaaagaaacgatcagacagatctggaatg
tgaagcgttatagaagataactggcctcatttcttcaaaatatcaagtgttgggaaagaaaaaaggaagtgg
aatgggtaactcttcttgattaaaagttatgtaataaccaaatgcaatgtgaaatattttactggactcttt

→ **a**

tgaaaaaccatctagtaaaagactggggtggggtgggaggccagcacggtggtgaggcagttgagaaatt
tgaatgtggattagatttgaatgatattggataattattggtaattttatggcctgtgagaagggtgttgt
agtttataaaagactgtcttaatttgcatacttaagcatttaggaatgaagtgttagagtgtcttaaaatgt
ttcaaatggtttaacaaaatgtatgtgaggcgtatgtggcaaaatgttacagaatctaactggtggacatgg
ctgttcattgtactgtttttttctatcttctatatgtttaaaagtatat**aataaa**atattta

Figure 10 (Continued)

gatctgccttccttcctgcccccatgtttgtctttccttgtttgtctttatatagatcaagcaggttttaaa 72
ttcctagtaggagcttacatttacttttccaaggggggaggggggaataaatatctacacacacacacacacac 144
H4TF-1 GH
acaccacactggagttcgagacgaggcctaagcaacatgccgaaaccccgtctctactaaatacaaaaaata 216
DTF-1
gctgagcttggtggcgcacgcctatagtcctagctactggggaggctgaggtgggaggatcgcttgagccca 288
Sp1
agaagtcgaggctgcagtgagccgagatcgcgccgctgcactccagcctgagcgacagggcgaggctctgtc 360
tcaaaacaaacaaacaaaaaaaaaaaggaaaggaaatataacacagtgaaatgaaaggattgagagaaatga 432
β-IFN HiNF-A
aaaatatacacgccacaaatgtgggagggcgataaccactcgtagaaagcgtgagaagttactacaagcggt 504
cctcccgggcaccgtactgttccgctcccagaagccccgggcgccggaagtcgtcactcttaagaagggacg 576
AP-2 E4F1
gggccccacgctgcgcacccgcgggtttgct ATG GCG ATG AGC AGC GGC GGC AGT GGT GGC 637
M A M S S G G S G G

Figure 11

cggcgtggtagcaggcc ATG GCG ATG GGC AGT GGC GGA GCG GGC TCC GAG CAG GAA 56
Met Ala Met Gly Ser Gly Gly Ala Gly Ser Glu Gln Glu

GAT ACG GTG CTG TTC CGG CGT GGC ACC GGC CAG AGT GAT GAT TCT GAC ATT TGG 112
Asp Thr Val Leu Phe Arg Arg Gly Thr Gly Gln Ser Asp Asp Ser Asp Ile Trp

GAT GAT ACA GCA TTG ATA AAA GCT TAT GAT AAA GCT GTG GCT TCC TTT AAG CAT 166
Asp Asp Thr Ala Leu Ile Lys Ala Tyr Asp Lys Ala Val Ala Ser Phe Lys His

GCT CTA AAG AAC GGT GAC ATT TGT GAA ACT CCA GAT AAG CCA AAA GGC ACA GCC 220
Ala Leu Lys Asn Gly Asp Ile Cys Glu Thr Pro Asp Lys Pro Lys Gly Thr Ala

AGA AGA AAA CCT GCC AAG AAG AAT AAA AGC CAA AAG AAG AAT GCC ACA ACT CCC 274
Arg Arg Lys Pro Ala Lys Lys Asn Lys Ser Gln Lys Lys Asn Ala Thr Thr Pro

TTG AAA CAG TGG AAA GTT GGT GAC AAG TGT TCT GCT GTT TGG TCA GAA GAC GGC 328
Leu Lys Gln Trp Lys Val Gly Asp Lys Cys Ser Ala Val Trp Ser Glu Asp Gly

TGC ATT TAC CCA GCT ACT ATT ACG TCC ATT GAC TTT AAG AGA GAA ACC TGT GTC 382
Cys Ile Tyr Pro Ala Thr Ile Thr Ser Ile Asp Phe Lys Arg Glu Thr Cys Val

GTG GTT TAT ACT GGA TAT GGA AAC AGA GAG GAG CAA AAC TTA TCT GAC CTA CTT 436
Val Val Tyr Thr Gly Tyr Gly Asn Arg Glu Glu Gln Asn Leu Ser Asp Leu Leu

TCC CCG ACC TGT GAA GTA GCT AAT AGT ACA GAA CAG AAC ACT CAG GAG AAT GAA 490
Ser Pro Thr Cys Glu Val Ala Asn Ser Thr Glu Gln Asn Thr Gln Glu Asn Glu

AGT CAA GTT TCC ACA GAC GAC AGT GAA CAC TCC TCC AGA TCG CTC AGA AGT AAA 544
Ser Gln Val Ser Thr Asp Asp Ser Glu His Ser Ser Arg Ser Leu Arg Ser Lys

GCA CAC AGC AAG [illegible] AAA GCT [illegible] CCG TGG ACC TCA TTT CTT CCT CCA CCA CCC 598
Ala His Ser Lys Ser Lys Ala Ala Pro Trp Thr Ser Phe Leu [illegible] Pro Pro Pro

CCA ATG CCA [illegible] [illegible] GGA TTA GGA CCA GGA AAG CCA GGT [illegible] AAA [illegible] AAC GGC 652
Pro Met Pro [illegible] [illegible] Gly Leu Gly Pro Gly Lys Pro Gly [illegible] Lys Phe Asn Gly

CCG CCG CCG [illegible] CCT CCA CTA [illegible] [illegible] [illegible] [illegible] [illegible] [illegible] [illegible] [illegible] [illegible] ATG CCC 706
Pro Pro Pro [illegible] Pro Pro Leu Pro Pro Pro [illegible] Phe Leu [illegible] Cys Trp Met Pro

CCG TTC CCT [illegible] [illegible] CCA [illegible] ATA ATC [illegible] CCA [illegible] [illegible] [illegible] [illegible] [illegible] CCC GAC 760
Pro Phe Pro [illegible] [illegible] [illegible] [illegible] Ile Ile Pro Pro Pro Pro [illegible] [illegible] [illegible] Pro Asp

TGT CTG GAT [illegible] [illegible] [illegible] [illegible] [illegible] GGC AGT ATG CTA [illegible] [illegible] [illegible] [illegible] ATG AGT 814
Cys Leu Asp [illegible] [illegible] [illegible] [illegible] Leu Gly Ser Met Leu Ile [illegible] [illegible] [illegible] Met Ser

GGC TAC CAT [illegible] [illegible] [illegible] [illegible] ATG GGT [illegible] AGA CAA AAT AAA AAA GAA GGA AAG 868
Gly Tyr His [illegible] [illegible] [illegible] [illegible] Met Gly Ile [illegible] Gln [illegible] Lys [illegible] Glu Gly Lys

TGC TCA CAT [illegible] 887
Cys Ser His [illegible]

Figure 12

```
 20        30        40        50        60        70        80
GSGGGVPEQEDSVLFRRGTGQSDDSDIWDDTALIKAYDKAVASFKHALKNGDICETSGKPKTTPKRKPAK

GSGGAGSEQEDTVLFRRGTGQSDDSDIWDDTALIKAYDKAVASFKHALKNGDICETPDKPKGTARRKPAK
         20        30        40        50        60        70
 90       100       110       120       130       140       150
KNKSQKKNTAASLQQWKVGDKCSAIWSEDGCIYPATIASIDFKRETCVVVYTGYGNREEQNLSDLLSPIC

KNKSQKKNATTPLKQWKVGDKCSAVWSEDGCIYPATITSIDFKRETCVVVYTGYGNREEQNLSDLLSPTC
         90       100       110       120       130       140
160       170       180       190       200       210       220
EVANNIEQNAQENENESQVSTDESENSRSPGNKSDNIKPKSAPWNSFLPPPPPMPGPRLGPGKPGLKFNG

EVANSTEQNTQENE--SQVSTDDSEHSSRSLRSKAHSKSKAAPWTSFLPPPPPMPGSGLGPGKPGLKFNG
        160       170       180       190       200       210
230       240       250       260       270       280       290
PPPPPPPPPPHLLSCWLPPFPSGPPIIPPPPPICPDSLDDADALGSMLISWYMSGYHTGYYMGFRQNQKE

PPPPPPLPPPPFLPCWMPPFPSGPPIIPPPPPISPDCLDDTDALGSMLISWYMSGYHTGYYMGFRQNKKE
220       230       240       250       260       270       280
300
GRCSHSL

GKCSHTN
290
```

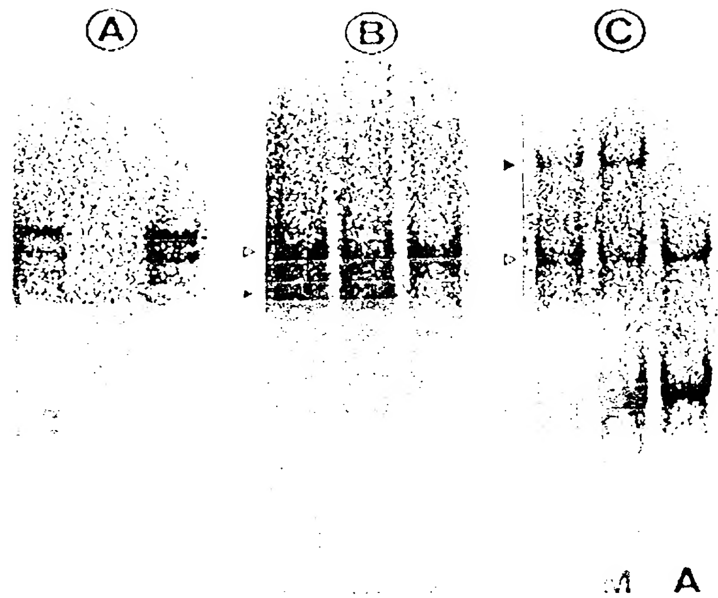


Figure 14

SSCP Analysis

SMN

C-BCD541

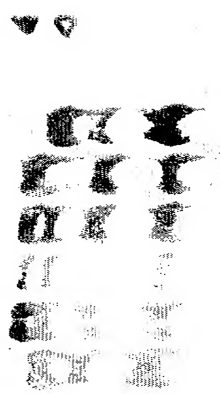


Figure 15

BIRCH, STEWART, KOLASCH & BIRCH, LLP

PLEASE NOTE YOU MUST COMPLETE THE FOLLOWING

COMBINED DECLARATION AND POWER OF ATTORNEY FOR PATENT AND DESIGN APPLICATIONS

ATTORNEY DOCKET NO. 2121-110P

As a below named inventor, I hereby declare that: my residence, post office address and citizenship are as stated next to my name; that I verily believe that I am the original, first and sole inventor (if only one inventor is named below) or an original, first and joint inventor (if plural inventors are named below) of the subject matter which is claimed and for which a patent is sought on the invention entitled:*

Insert Title: SURVIVAL MOTOR NEURON (SMN) GENE: A GENE FOR SPINAL MUSCULAR ATROPHY

Check Box If Appropriate - For Use Without Specification Attached

the specification of which is attached hereto unless the following box is checked:

☒ was filed on October 19, 1995 as United States Application Number 08/545,196 or PCT International Application Number ______ and was amended on October 19, 1995 (if applicable).

I hereby state that I have reviewed and understand the contents of the above identified specification, including the claims, as amended by any amendment referred to above.

I acknowledge the duty to disclose information which is material to patentability as defined in Title 37, Code of Federal Regulations, §1.56.

I do not know and do not believe the same was ever known or used in the United States of America before my or our invention thereof, or patented or described in any printed publication in any country before my or our invention thereof, or more than one year prior to this application, that the same was not in public use or on sale in the United States of America more than one year prior to this application, that the invention has not been patented or made the subject of an inventor's certificate issued before the date of this application in any country foreign to the United States of America on an application filed by me or my legal representatives or assigns more than twelve months (six months for designs) prior to this application, and that no application for patent or inventor's certificate on this invention has been filed in any country foreign to the United States of America prior to this application by me or my legal representatives or assigns, except as follows.

I hereby claim foreign priority benefits under Title 35, United States Code, §119 (a)-(d) of any foreign application(s) for patent or inventor's certificate listed below and have also identified below any foreign application for patent or inventor's certificate having a filing date before that of the application on which priority is claimed:

Insert Priority Information (if appropriate)

Prior Foreign Application(s)

(Number)	(Country)	(Month/Day/Year Filed)	Priority Claimed Yes	No
94402353.0	Europe	October 19, 1994	☒	☐
			☐	☐
			☐	☐
			☐	☐
			☐	☐

I hereby claim the benefit under Title 35, United States Code, § 119(e) of any United States provisional application(s) listed below.

(Application Number)	(Filing Date)

All Foreign Applications, if any, for any Patent or Inventor's Certificate Filed More Than 12 Months (6 Months for Designs) Prior To The Filing Date of This Application:

Country	Application No.	Date of Filing (Month/Day/Year)

I hereby claim the benefit under Title 35, United States Code, §120 of any United States application(s) listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States application in the manner provided by the first paragraph of Title 35, United States Code, §112, I acknowledge the duty to disclose information which is material to patentability as defined in Title 37, Code of Federal Regulations, §1.56 which became available between the filing date of the prior application and the national or PCT international filing date of this application:

(Application Number)	(Filing Date)	(Status — patented, pending, abandoned)

*NOTE: Must be completed

I hereby appoint the following attorneys to prosecute this application and/or an International application based on this application and to transact all business in the Patent and Trademark Office connected therewith and in connection with the resulting patent based on instructions received from the entity who first sent the application papers to the attorneys identified below, unless the inventor(s) or assignee provides said attorneys with a written notice to the contrary:

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I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Full Name of First or Sole Inventor: Insert Name of Inventor Insert Date This Document Is Signed

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Full Name of Fourth Inventor, if any: see above

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Full Name of Fifth Inventor, if any: see above

GIVEN NAME FAMILY NAME	INVENTOR'S SIGNATURE	DATE*

Residence (City, State & Country)	CITIZENSHIP

POST OFFICE ADDRESS (Complete Street Address including City, State & Country)

*Note: Must be completed date this document is signed